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PRELIMINARY OBSERVATIONS ON THE STRUCTURE OF THE UTERUS AND THE PLACENTA OF A FEW INDIAN ELASMOBRANCHS

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Introduction

VIVIPARITY among Elasmobranchs is by no means uncommon. As stated by E W Shann (1923) three general methods of nutrition of the young may be said to be in vogue Either the yolk-sac forms a pseudo-placental connection with the uterine wall as in *Scoliodon sorrakowah* (Fig 1), *Scoliodon walbeehmi* (Photograph 1), *Scoliodon palasorrah* (Photograph 2), *Hemigaleus balfouri* and *Mustelus lævis*, or the uterine wall secretes a nutrient fluid which is absorbed by means of external gill filaments, or again, the uterine wall itself is produced into long secretory villi or papillæ, which enter the alimentary canal of the embryo by way of the spiracles

According to the mode of obtaining nourishment certain structures are developed by the parent fish as well as by the embryos during the different periods of intra-uterine existence In the placental forms as in *S palasorrah* and *S walbeehmi*, in the earlier embryonic stages, a placenta is absent, the yolk in the yolk-sac being the main source of nourishment But as pregnancy advances the yolk gets absorbed and a placenta is developed by the modification of the yolk-sac and then nourishment is obtained through the blood vascular system.

In *Carcharinus dussumieri* no yolk-sac placenta is at all developed due to the large amount of yolk in the yolk-sac which provides nourishment for the growing enbryos during a greater part of their intra-uterine development. In *S. sorrakowah*, on the other hand, the yolk present being very poor, the embryo is compelled to obtain nourishment by other ways and so the placenta is established very early in development

In the placental Elasmobranchs, the Rays and Skates, the yolk-sac persists, the yolk being taken directly into the alimentary canal In some cases an internal yolk-sac is also present. The blood vessels in the mesoblastic portion of the yolk-sac are also of use in absorbing the nourishment contained

within it. External gill filaments present in the early stages of the embryos also help in absorption Southwell and Prashad (1919) have mentioned that in some forms certain special processes, the trophonemata, are developed which enter the embryonic spiracles and pour the secretion into the pharynx.

In most species of *Scoliodon* special structures, the appendicula, are developed on the placental cord as in *S sorrakowah* (Photograph 3), *S palasorrah* (Photograph 2), *S walbeehmi* (Photograph 1) These are simple, long and filamentous and rarely branched in *S sorrakowah*, much branched in *S palasorrah*, very short and branched in *S walbeehmi* and entirely absent in *Carcharinus dussumieri* (Photograph 4) The function of these is to help in the absorption of the uterine secretion

In the sharks the different parts of the oviduct are functionally modified, for, although the ova are fertilized within the oviduct, the development of the young is not carried out in invariably the same plan, some being fully developed and born alive, while in others the ova are encased in a horny covering and deposited in the sea, where they undergo a protracted development.

Historical Account

A short historical account of the studies of Elasmobranch fœtus and placenta may not be out of place here But the account which follows cannot be regarded as being complete

Aristotle —The placenta of *Mustelus lævis* was first described by him. But his discovery was not paid any attention to till 1842 when John Muller once more described the placental connection

Pierre Belon (1533) and Guillaume Rondelet (1554) knew of the attachment of the fœtus to the uterus of the mother in some Elasmobranchs

Home (1810) describes the anatomy of the oviduct of *Acanthias vulgaris*

Cuvier (1829) in his work on fishes mentioned that in *Carcharias* the yolk-sac and uterus are attached in the form of a placenta

John Davy (1834) describes longitudinal ridges in the gravid uterus of *Torpedo marmorata* and papillæ in that of *T oculata* The occurrence of uterine fluid with a nutritive function has also been mentioned by him. He stresses the absence of a vascular connection between the embryo and the mother.

John Muller (1840) worked on *Centrophorus granulatus* and observed that this species builds a long series of small, triangular lobe-like papillæ as in *Acanthias* In *Scymnus lichia* he describes cylindrical papillæ set in longitudinal rows. According to Brinkman (1903–4) these papillæ are arranged

in double rows. He was also the first to give the anatomical description of the placenta of *Mustelus lævis* He has also left his observations on the fœtus of *Spinax niger, Torpedo oculata,* etc

Leydig (1852) states that in the mucosa of the uterus of *Squalus acanthias* are found well-developed tufts which stand in definite rows and pass into leaf-like folds towards the end of the uterus He has also given a short description of the placenta of *Mustelus lævis*

Bruch (1860) studied *Squalus acanthias* and mentions that there is co-ordinate growth between the tufts, uterine walls and the embryo.

Trois (1867) studied *Squalus acanthias* and observed the circulation in the uterine tufts

Ercolani (1879) investigated the viviparous selachians from the standpoint of relationship between the embryo and the uterus and on this relationship classified them under four groups according to Muller .

(i) Contact between the uterine wall and the fœtal wall, smooth (*Plagiostomi acotyledonale di Muller*)

(ii) Complicated contact between the two surfaces, and a great increase in the secreting surface (*Idem*)

(iii) Contact distinguished by a new development of tufts, villi, etc. (*Alcuni selaci*)

(iv) Intimate contact and union on the part of the absorbing region with another part of the secreting surface (*Plagiostomi cotyledonale di Muller*)

Mehrdorf (1890) describes the histological condition of the yolk-sac of *Mustelus.*

Brinkman (1903) studied the structural changes of the uterus of a few sharks and rays He made a comparative study of the pre-pregnant and pregnant stages of *Squatina angelus* and describes the changes that take place in the epithelial cells of the uterus.

Borcea (1905) has given an excellent account of the structure of the uterus in a number of European Elasmobranchs.

Widakowich (1907) worked on the uterus of *Squalus acanthias* in detail and also dealt with the developmental history of other sharks He places *Squalus acanthias* under the second group of Ercolani's classification of the selachians The uterus is supplied by two systems of vessels, the organotrophic and the embryotrophic. The uterine arteries are protected against compression during egg-passage by the development of venous pockets.

Gudger, E. W. (1912 *b*) made observations on the uterus and the intra-uterine embryo of *Sphyrna tiburo*. He states that the inner mucous lining of the uterus is separated from the outer muscular coat by a fibrous spongy material which allows considerable growth of the embryo before any distension of the outer wall is necessary Each embryo with its yolk-sac is contained in a very thin, tough, elastic, highly iridescent membrane. The end of the shell, he says, is curious in being plaited and folded, the purpose of which is to accommodate the growing embryo

Gudger (1912 *a*, *c*, 1914, 1915) has also contributed other papers on the life history of Elasmobranchs to which reference may be made

Shann, E W (1923) studied the embryonic development of *Lamna cornubica* In this he found the mode of nourishment very different from those of other sharks The original yolk-sac gets absorbed at a very early period. The nutrient material is derived from the ovary in the form of immature eggs or partly degenerate ovarian tissue which are taken up by the oviduct and passed into the uterus where they are swallowed by the embryos Feeding like this continues for a long time, over a year, when the cardiac-stomach assumes gigantic proportions and is called the yolk-stomach He believes that this food is not used for body-building but for the building up of the reproductive organ

Tencate-Hoedemaker (1933) gives a detailed description of the placenta of *Mustelus lævis* The placenta according to him is divided into two parts: the maternal and the fœtal The maternal placenta is built out of strongly branched, leaf-like out-pushings of the uterine wall, the villi, which are covered by the embryonal tissue He compares the placenta of this with that of viviparous reptiles and of mammals

With regard to the Indian forms with which we are more directly concerned it may be said that very little work has been done

Alcock (1890) made observations on the gestation of some of the Indian Sharks and Rays In *Carcharias melanopterus* each embryo is lodged in a compartment and the placental cord, due to branching, forms a compact arborescent mass which attaches itself to a flat vascular disc on the wall of the uterus, thereby forming the placenta The placental cord in *Zygæna blochi* is devoid of appendicula The uterine mucosa of *Trygon bleekeri* is thrown into thick set papillæ which secrete the uterine milk and there is no connection between the fœtus and the mother In *Myliobatis nieuhofii*, he states that the uterine papillæ are less attenuated and the whole intra-uterine mucosa forms a superficial milk gland. His other papers (1892) on the fœtus of other elasmobranchs are also of considerable interest.

In 1891 Wood Mason and Alcock published their " Further observations on the gestation of Indian Rays". The forms dealt with by them are *Pteroplatæa micrura, Myliobatis nieuhofii* and *Trygon walga* of which the last has been studied in detail In these batoids they found that the elongated papillæ of the uterus are beset with tubular glands which secrete a nutritive fluid and they believed that the glands developed are for the special requirements of the pregnant state

Southwell (1910), in *Pristis cuspidatus,* described the external walls of the uterus to be highly muscular and the internal walls to present the appearance usual among the viviparous selachians

In their paper Southwell and Baini Prashad (1919) have given a description of the intra-uterine embryos of some Indian Sharks and Rays, together with a discussion on various points of general interest based on a study of the yolk-stalk, appendicula and placenta in several sharks. They divide the appendicula into four types, tracing a complete series in the evolution of the long, thread-like, single and branching appendicula from mere projections on the wall of the placental cord They also distinguish three distinct grades in the development of the placenta in the forms dealt with by them

Thillayampalam (1928) states that in *Scoliodon* when the yolk from the yolk-sac gets absorbed, the sac gets greatly folded and embeds itself in the uterine wall forming a placenta She believes that in forms with the best developed appendicula, the placenta is of the most primitive type thereby pointing out that the forms with a less highly organised type of placenta require some other mode of absorption of food

It will be seen from a perusal of the history of these studies that very much more attention has been paid to the uterus and the uterine structures than to the actual placental features except in *Mustelus lævis* where the placenta has been studied in detail

Material and Methods

The period of collection extended from August 1936 to August 1937. The material for study includes specimens of *Scoliodon sorrakowah, S palasorrah, S. walbeehmi* and *Carcharinus dussumieri.*

The fixatives employed have been of several kinds The most satisfactory was formalin especially when slightly stronger than 5% The other fixatives used were Bouin's fluid, absolute alcohol, Zenker's formal, Corrosive acetic-formol and Carnoy The material was dehydrated and then cleared either in Cedarwood oil or Xylol in the usual manner. In all cases

paraffin method of embedding was employed and sections cut were seven to ten microns thick. Iron hæmatoxylin followed by Eosin or Van Gieson was found to give good results. Other stains used were Delafield's hæmatoxylin, Mallory's triple stain, Thionin blue and Best's carmine.

PART I—SCOLIODON SORRAKOWAH

Reproductive System

(Fig 2)

The female specimens examined measured between 18·5" and 29". The ovaries are paired structures, lying behind the base of the liver They are attached posteriorly to a pair of epigonal organs which are in the form of long strands of tissue In all cases examined, the ovary was very small with very inconspicuous eggs The ova were no bigger than those of a frog, in fact, were even smaller, whereas in the other Elasmobranchs that I have observed the ova grow to a much larger size This small size of the ova in *S. sorrakowah* is characteristic, and has not been observed before Kerr (1919) says that the egg of the Elasmobranch at the time immediately preceding gastrulation differs from the blastula of the ordinary amphibian or lung fish in its much greater size This is probably true for most of the Elasmobranchs In *S sorrakowah* the mature eggs are undoubtedly small I first believed that the smallness of the eggs was due to a fresh set of eggs being under development, but on careful examination found that there was hardly any difference in size of eggs in specimens measuring from 18·5" to 29" containing embryos varying from 20 mm to 135 mm in length, between which stages there must have elapsed a period of at least five to six months Even in the two non-pregnant specimens from Malabar the ovary contained inconspicuous eggs The above, taken in conjunction with the fact that the yolk-sac in the embryo measuring 20 mm, the smallest obtained, was very small, about 4 mm, containing very little yolk, proves that in *S sorrakowah* the eggs are actually very small and their early development is greatly influenced thereby.

The oviducts consist of a pair of tubes which extend along the whole length of the body cavity Anteriorly these oviducts converge towards the middle line and open into the cœlom by a single, median, longitudinal slit, the oviducal funnel. At the anterior one-third, the oviducts dilate to form the shell glands which are rather small, and measure usually about 4 mm in length and 3 mm. in width

An interval of 10 to 11 mm follows between the hind end of the shell gland and front end of the uterus in the early pregnant uterus but in advanced pregnancy the interval becomes slightly less.

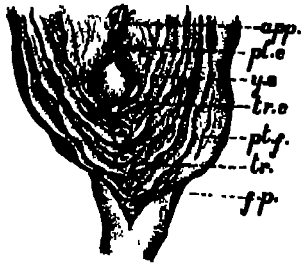
The caudal part of each oviduct dilates to form the uterus in which the young are developed During the breeding season due to the growing embryos the uteri distend and occupy the greater part of the abdominal cavity Embryos grow to a large size, as much as 135 mm , before being born The uteri finally join together and open into the cloaca.

The Pregnant Uterus

The young pregnant uterus is spongy, thick-walled and well-vascularised As pregnancy advances, the uterine wall loses its spongy nature and elasticity but becomes more vascular and due to the very great stretching, the wall shows as a thin, smooth, semi-transparent vascular membrane That blood supply is greater in viviparous forms, than in oviparous is well seen in *S sorrakowah* when compared with *Chiloscyllium*, an oviparous form The thickness of the wall depends on the period of gestation, the more advanced the period, the thinner the wall In this general thinning of the uterus that side of the uterus against which the dorsal surface of the embryo is pressed, undergoes the maximum stretching The posterior end of the uterus remains thick and highly vascularised even in the later periods of pregnancy

Each uterus is divided into as many compartments as there are embryos These compartments lie longitudinally, slightly one behind the other, and in a transverse section at least three of them are found to run side by side (Photomicrograph 5 and Fig 3) The arrangement is easily made out in the early stages but as pregnancy advances the compartments lengthen out to a great extent and run along the whole length of the uterus and only on examination of the posterior end of the uterus, can it be seen that each compartment is placed slightly one behind the other (Fig 1)

I believe that here as in *S palasorrah* to be described later, the partition walls are formed by two folds of the uterine mucosa coming together and establishing intimate connection between the epithelia Hence in the early stages the compartments are more or less obliquely transverse (Fig. 4) and later due to stretching and unequal growth of the uterine and partition walls come to be arranged in a longitudinal manner. The walls are thick and spongy at first but later become so thin and delicate, that unless the uterus is carefully opened, they rupture and shrink in places to such an extent that they give the impression of mere ridges of the inner uterine wall (Fig 1)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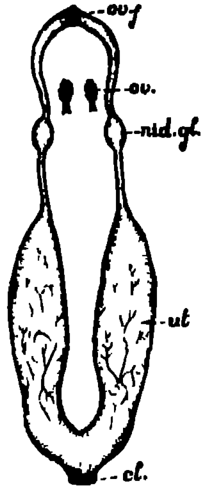
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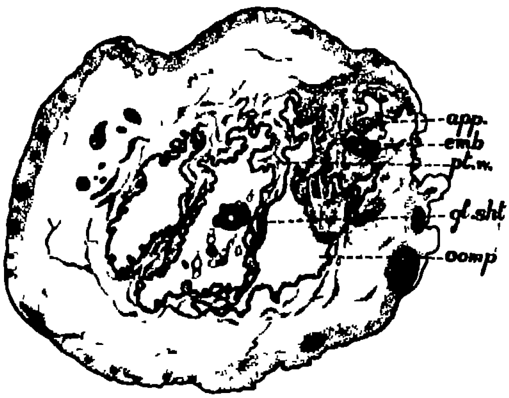
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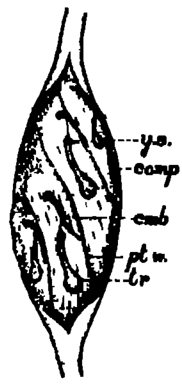
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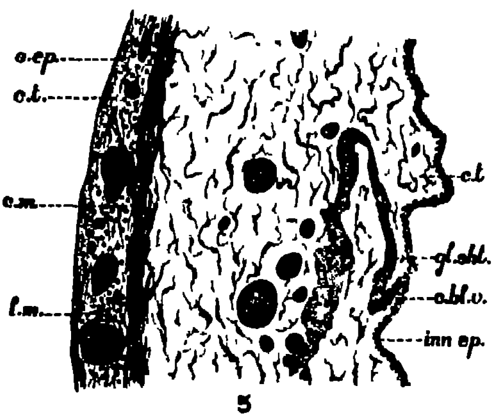
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At the hind end of each compartment the internal mucous membrane is raised up into a finger-shaped process, the trophonema* (Photomicrograph 6 and Fig 1) Here the uterus is characterised by a rich and congested blood supply.

The number of embryos found in each uterus is not constant and may vary from one to five, only in one case have I found six embryos. In most uteri, even in those containing one or two embryos, I have noticed five trophonemata and only in one case a sixth trophonema was observed, that is in the uterus with six embryos This probably shows that five is the usual number developed though at the time of capture the number may be reduced by premature birth, due to shock and probably also to one or two undergoing aborted development Even the number of embryos in the right and left uteri may differ though generally they are about equal The majority of the embryos have been observed with their heads towards the cranial side of the mother and a few towards the caudal side They lie with their tails bent forwards to one side The placental cord runs between the pectoral knobs of the fœtus and is continued into the yolk-sac

The size of the smallest embryo obtained was 20 mm (Photomicrograph 7) and the biggest 135 mm Each compartment is filled with uterine fluid which surrounds and protects the embryo and is also nutritive in nature Each embryo is completely enveloped in a thin, delicate shell-membrane secreted by the reduced shell gland. This membranous bag is found to be folded and twisted at the anterior end and lies free in the lumen This part gradually stretches out to accommodate the growing embryo

Detailed Structure of Pregnant Uterus—Early Condition (Fig 5)—As already mentioned, the uterus, in the early stages of pregnancy is found to be spongy and thick-walled It presents the following structure in a transverse section —

1 An outer thin, epithelial layer of more or less flattened, easily staining cells, one cell deep, with flattened nuclei At the posterior end of the uterus where no great distension takes place, the cells are rounded with deeply staining, ovoidal nuclei.

2 A thin, serous layer of connective tissue.

3 Muscular layers, longitudinal and circular, between which run large thin-walled blood vessels The circular muscle layer is thicker than the longitudinal

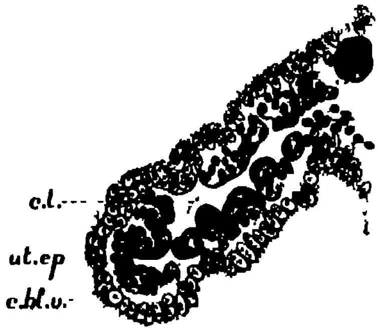
* The terms ' trophonema ' and ' trophonemata ' are used throughout the paper in the sense they were used by Woodmason and Alcock.

4. A sub-mucosa layer with loose connective tissue Numerous spaces occur in between the tissue which give the wall a loose and spongy appearance. This layer is well vascularised and here and there strands of muscles occur In this part of the wall of the uterus, very often we come across a sheet-like structure made up of glandular cells This sheet is present in the partition walls of the compartments as well (Fig 3) Where the epithelia come together the cells get differentiated to form the glands The outlines of these gland cells could not be made out, but the nuclei are small and scattered with no definite arrangement The function of these glands is probably secretory. A very interesting feature, noticed next, are the numerous blood vessels which occur directly under the epithelium (Photo-micrograph 9 and Fig 6) These vessels are in the form of a net-work and this is a very characteristic feature in the early stages of pregnancy The basement membrane which separates the inner epithelium from the connective tissue layer, in non-pregnant uteri usually, could not be made out here.

5. Lastly, there is an inner epithelium which is one-layered (Fig 6). The cells of this layer are of a uniform size over the entire surface of the compartment and are small, cubical, possessing ovoidal or rounded nuclei, but the cell boundaries have become indistinguishable These cells are glandular in nature and pour the secretion into the lumen Here and there tips of cells along with the nuclei are found to be cut off and thrown into the lumen along with the secretion

The secretion does not answer the test for mucin and is probably of a nutritive nature intended for the nourishment of the embryos The absorption of the uterine fluid is believed to be usually by the external gill-filaments, but this fluid has been found in the compartments at a stage when the filaments are in the form of mere knobs The presence of a non-ciliated epithelium is a characteristic feature not only of this form but also of *S. palasorrah*, and Leydig (1852) and Widakowich (1904) also mention the absence of cilia in *Acanthias*

Structure of Uterus—Advanced Condition (Fig 7)—As pregnancy advances, the outer epithelial cells get less compactly arranged and become greatly flattened, so also the nuclei The layer of connective tissue with numerous connective tissue fibres following the epithelium is now very much better developed The longitudinal muscles below have undergone reduction The circular muscles remain unaltered and the blood vessels occurring in between the layers are now very well developed The sub-mucosa undergoes marked reduction over its whole extent but there is an increase in the number of blood vessels though the chain of vessels characteristic of the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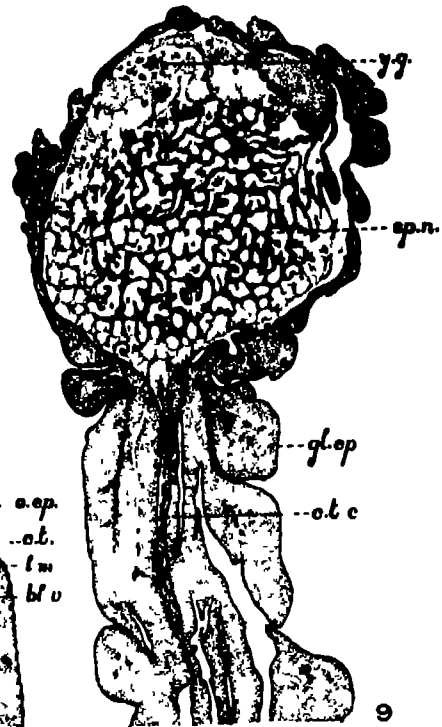
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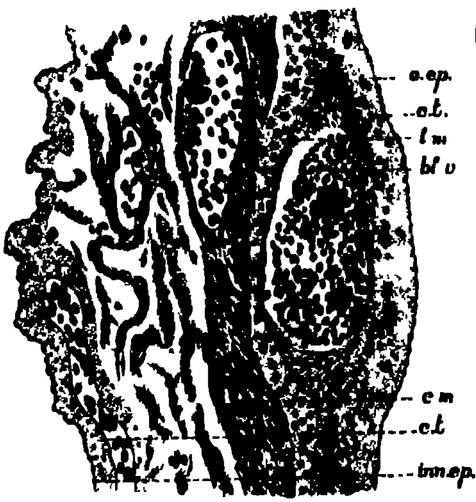
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sub-epithelial region of the early pregnant uterus is now absent These changes are accompanied by the degeneration of the uterine epithelium The epithelium is now present in the form of a single layer of flattened, rather loosely arranged cells In the course of pregnancy the epithelium appears to be completely peeled off in certain places, and regeneration probably, takes place later.

Structure of Uterine Partition

(Photomicrograph 9 and Fig 8)

The partition walls consist mainly of connective tissue bounded on both sides by epithelium As already mentioned, in each partition there is a sheet-like structure made up of gland cells described here, I believe, for the first time The cells of the sheet resemble closely, the inner epithelial cells of the uterus. In all sections these can be traced to the inner epithelium As in *S palasorrah*, to be described in Part II, I believe that here also the partition wall is formed by two folds which overlap each other in the very early stage. Later, where the epithelia of the two folds meet, the cells get differentiated and become glandular The outlines of the cells in *S sorrakowah* are not distinguishable In *S palasorrah* as will be pointed out more fully later, the glands are tubular and very much better developed In *Mustelus lævis*, Hoedemaker finds that the outer surface of the septa is not entirely smooth but carries additional secondary septa Such secondary septa are absent in *S sorrakowah.*

Trophonemata and Yolk-sac—General

The term trophonemata was applied, in the first instance, by Wood Mason and Alcock to the extensions of the mucous membrane of the uterus in *Pteroplatea micrura* In *S sorrokowah* these structures are described for the first time and as many trophonemata are present as there are embryos The trophonemata are developed in the posterior half of the uterus, one at the base of each compartment These are thick, solid structures with a very much fissured, rough, glandular outer surface Each measures about 12 to 15 mm in length and 4 mm in width at the base, in the early stages of gestation (Fig 4). Trophonemata, in general, are special processes of the uterine mucosa with a definite function They have different shapes, not only in the various forms but also in the various stages of development of the same form In the early stages of gestation, each trophonema is a cord-like structure narrow at the proximal end and gradually widening towards the distal end. Here it invaginates to form a cup, into which the yolk-sac fits and is almost completely enveloped, establishing a firm connection

between the two in the early stages Thus a yolk-sac placenta is formed (Photomicrograph 10 and Fig. 9) As pregnancy progresses, the yolk-sac grows in size and the cup becomes less and less deep So that it begins to loose its firm hold on the sac (Fig. 1) The yolk-sac gets dislodged gradually. In the final stages, the trophonema becomes shorter and broader and is no longer a solid structure, the core now being more or less hollow. The outer surface is now smooth The trophonematous cup has become broader and more shallow The rim of the cup is now thrown into finger-shaped processes forming a crown The yolk-sac also is produced into similar processes at its distal end The connection between the trophonema and the yolk-sac has become loose and a mere touch is enough to dislodge the sac from the cup Such processes on the yolk-sac have been observed by Southwell and Prashad (1919) though no mention of the trophonemata is made by them. The trophonemata probably get completely absorbed in the final stages. Progressive stages of the reduction of trophonemata have been noticed, but in no specimen examined have I found them entirely absent It has been observed, that there is co-ordinate growth between the trophonemata, uterus and the embryos and also a relation between the period of pregnancy and the length and thickness of the trophonema

Structure of Trophonemata —In a transverse section of the uterus through the posterior end of the compartment, the outer wall of the trophonema can be seen in continuation with the epithelium of the uterus.

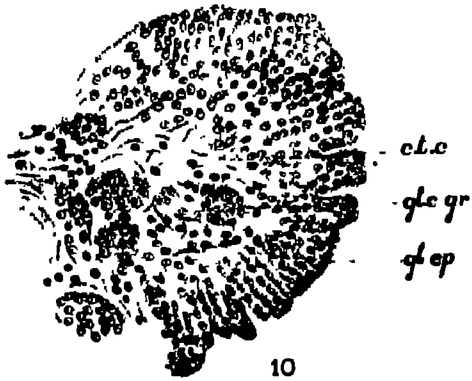
In early pregnancy, a transverse section (Photomicrograph 6) shows the trophonemata occupying the greater part of the lumen Sections, both transverse (Photomicrograph 11) and longitudinal (Photomicrograph 10 and Fig 9) through a young trophonema show a narrow central core of connective tissue and a broad outer glandular region This outer rind is made up of numerous, deep folds of the uterine epithelium into which the sub-mucosa penetrates (Photomicrograph 12 and Fig 10) The epithelium of the folds consists of several irregular layers of cells, the outermost being made up of long pointed cells with large granulated, rounded, terminal nuclei The nuclei of some of these are very chromatic The gland cells nearer the core are grouped together having got into a deeper position and thereby getting isolated from the outer glandular layer. The core of the trophonema at the base is made up of thick connective tissue with numerous blood vessels. The connective tissue can be seen throughout, to run in between the groups of gland cells of the outer region In the core individual free cells with conspicuous deeply staining nuclei and little protoplasm are found along with leucocytes It is difficult to account for the presence of the free cells in this region unless they be regarded as degenerate cells which have detached

themselves from the outer glandular rind Wood Mason and Alcock (1891) mention the ocurrence of these in the papillæ of *Trygon walga* and regard them as degenerate cells thrown out from the glands They consider the leucocytes to have a resorptive function

Condition of the Trophonemata in Late Pregnancy —As pregnancy advances, the outer glandular region becomes attenuated and loses its deep folds. The number of cell layers is reduced to three, two or even one (Fig 11) The outermost layer has long cells projecting here and there into the lumen of the uterus. In the greater part, however, cells are small and round with a continuous outline The outermost region of the core immediately under the glandular epithelium has become very vascular But for the narrow outer rind of gland cells the rest of the trophonema is made up of very well vascularised connective tissue More free cells now appear in the body of the trophonemata due to the degeneration of the glands All these changes are indicative of functional degeneration that the trophonemata have undergone.

L. S of Trophonema and Yolk-sac —Photomicrograph 10 and Fig 9 represent a longitudinal section of part of a young trophonema and yolk-sac. It will be seen that the yolk-sac is almost enveloped by the cup-like invagination of the distal end of the trophonema Fig 12 shows part of the cup and yolk-sac in a highly magnified condition. Externally there is the outer rind of gland cells Next is the thin laminal epithelium which in the invaginated part of the trophonemata consists of almost columnar cells with distinct nuclei Internal to this layer is the outer epithelium of the yolk-sac consisting of granulated cells This layer and the columnar cell layer run very close together and can be traced deep down into the bottom of the cup where the columnar cells become very conspicuous and the relation between the two epithelia, that of the yolk-sac and of the trophonema become much more intimate The nuclei of the columnar cells at the base of the cup are long and the cells themselves taper towards the trophonematous core and are very protoplasmic. Next to the epithelial layer of the yolk-sac is a layer of large rounded cells with prominent nuclei, the mesoderm Internal to this, is a layer of flattened cells, the endoderm These last two layers in places can be seen to be continuous with the chain of spongy cells forming the network inside the yolk-sac At this early stage yolk granules are found to be scattered all over, but confined mostly to the peripheral region

In still later stages (Photomicrograph 14), the cup presents the same structure as above except that the glandular rind is less conspicuous and the columnar cells more prominent (Photomicrograph 14 and Fig 13). The trophonema besides acting as a means for transferring nourishment through



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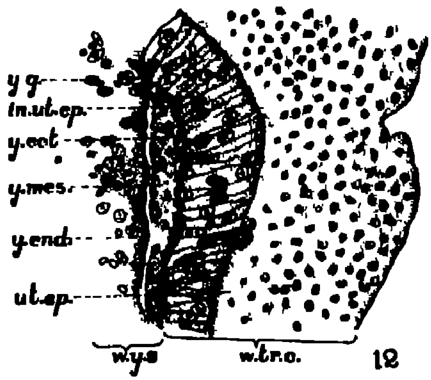

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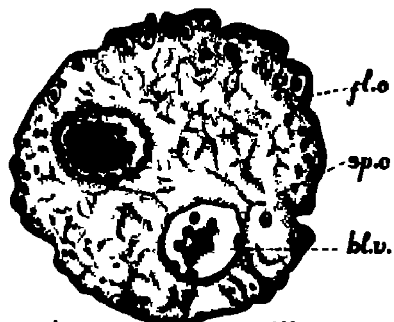

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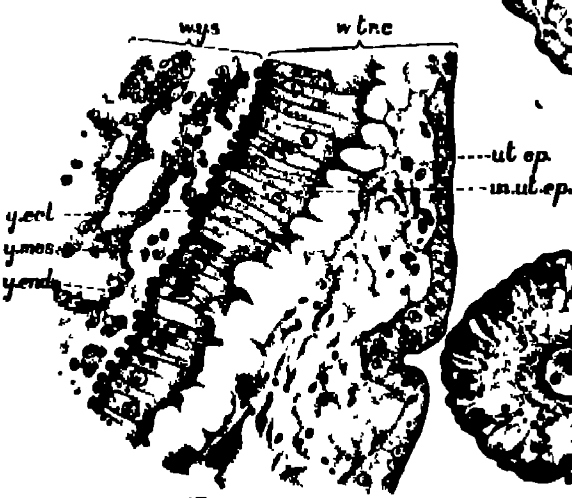

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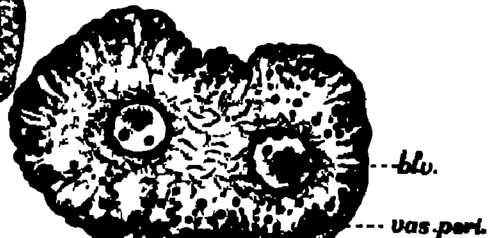

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its vascular system, secretes, judging from the development of gland cells on the trophonemata, a nutritive fluid

Yolk-sac

The study of the yolk-sac in this form is very interesting In *S. sorrakowah*, unlike in other elasmobranchs, the yolk-sac, to begin with, is very small due to the presence of very little yolk in it. Early establishment of a placental connection therefore becomes necessary In an embryo 20 mm long the yolk-sac was about the size of a pin-head and was already connected with the uterus (Fig. 4) I was not able to examine still earlier stages of embryos measuring less than 2 cms and hence cannot say how long the yolk-sac is free and at what stage the placenta is formed

The yolk-sac placenta is made up of the spongy yolk-sac and the trophonematous cup (Photomicrograph 10) It might, perhaps, be well to stress the fact that although the fœtal tissue is attached to the maternal tissue, there is no invasion of the maternal by the fœtal tissue The connection between the two does not last long, as the yolk-sac, as distinct from other forms, grows much bigger in size with the growth of embryos and hence the trophonematous cup is unable to have a firm hold on it. This increase in size of the yolk-sac is due to the increase in the absorptive surface (Photomicrograph 13 and Fig. 1). The proximal half of the yolk-sac at this stage is red and the distal half more or less white (Photograph 3) No difference is noted in the structure of the two regions

An important feature of the yolk-sac of *S sorrakowah* which I believe is mentioned here for the first time, is that the inside of the organ is not filled with yolk granules, as in the case, for instance, in *Pristis*, *Narcine*, *S. palasorrah*, *C dussumieri*, etc , but is filled up with a net-work of spongy tissue consisting of tubular cellular strands, blood vessels interspersed with connective tissue (Photomicrographs 10 and 13). One would have expected a free hollow yolk-sac here also but I have not been fortunate enough to obtain this stage. On the other hand it is not improbable that this stage has been completely lost due to the almost total loss of yolk giving rise to a highly specialised vascular structure which comes into early contact with the wall of the uterus. This deviation from the normal structure can only be attributed to the necessity for the development of very early connection between the embryo and the mother making transmission of nutrient material possible. The tubular strands repeatedly branch to form a regular net-work. In the early stages, the net-work is loose with few large spaces (Photomicrograph 15). The cells are rounded with conspicuous nuclei, though the cell-boundaries

are often difficult to make out. Numerous blood vessels and connective tissue occur between the chains of cells.

In later stages the mesh-work has become more compact. The intervening spaces have become very much smaller There are large blood vessels here and there (Photomicrograph 16). In the connective tissue numerous round free cells occur The structure of the yolk-sac attains its highest stage of progressive histological differentiation The rich supply of blood vessels makes the yolk-sac resemble a sponge saturated with slowly circulating blood On careful examination it is found that corpuscles of various sizes occur. It is possible that erythropoiesis takes place in the yolk-sac region.

Placental Cord

The term yolk-stalk is not applicable here since in the youngest stage obtained the placenta had already developed The structure is therefore referred to here as the placental cord During the period when the yolk-sac is free, direct communication must exist as pointed out by Southwell and Prashad between the yolk-sac and intestine, even in forms that later on have a placental arrangement No such stage has been observed in *S sorrakowah* by me. The placental cord is a thread-like structure in the very early stages which gets thicker and thicker as pregnancy progresses. This cord gives off numerous appendicula.

A transverse section of a placental cord in the very early stage shows that it is slightly flattened along two sides. On the outside is a layer of loosely arranged cells, projecting almost freely from the surface of the cord These cells have prominent nuclei with rather dense chromatin clumps Next is a layer of flattened cells placed far apart, following this is a layer formed of a large number of spongy cells almost forming a mesh-work The centre of the placental cord is occupied by two blood vessels, having very definite walls.

In the next stage observed the outer cells have disappeared leaving the flat cells exposed There is now a richer supply of blood vessels in the peripheral region while in the centre the two blood vessels, already mentioned, have increased in size. The cord of the embryo persists till a very late stage Only in one case did I notice the absence of placental cord and the umbilical scar left was still fresh Whether this was due to rough handling of the parent fish cannot be said, as progressive stages of its reduction have not been observed.

Appendicula

Alcock (1890) was the first to give an account of these structures. Appendicula are present in *S. sorrakowah*, *S. palasorrah* and *S. walbeehmi* and absent in *C. dussumieri*.

In *S. sorrakowah* these appendicula are numerous, simple, delicate, elongated, thread-like, well-developed structures Very few are found to be forked. Since they are long some are found embedded in the folds of the uterus and give the appearance of having established tissue connection with the uterine wall As pregnancy advances they become thicker, longer and very vascular The function of these appendicula is absorption

In the youngest embryo available an appendiculum in a transverse section (Fig 14), is found to be solid with a core of spongy cells and a few scattered blood corpuscles here and there, a layer of flattened cells surrounding it and an outer rind of highly protoplasmic columnar cells, the tips of some of which have an amœboid appearance In a more advanced stage (Fig 15), there is a compact spongy core, traversed longitudinally by two large blood vessels one being an artery and the other a vein The outer rind of columnar cells is lost Here and there a few tiny blood capillaries are found to be cut in the outer region of the core

In a still later stage the appendiculum gets flattened along two sides (Fig. 16). There is a central core of well-developed connective tissue, traversed longitudinally by two blood vessels Surrounding the core of connective tissue is a peripheral zone of capillaries The extraordinary development of the blood capillaries at the periphery goes to show that appendicula are getting modified to fulfil their function of absorption.

Alcock, with some doubt, considered the appendicula to be of the nature of lymphatic glands provided the channels of the placental cord be considered as lymphatics Southwell and Prashad state that the structure of the appendicula tends to show that they might, like villi, serve in absorbing the food material secreted by the uterine wall of the mother They distinguish four types of appendicula, the most primitive being in the form of small flat processes, the next in the form of tubular processes as in *S walbeehmi*, the third, more highly evolved, in the form of elongated much branched structures and lastly, the most highly-evolved type, in *S sorrakowah* and *S palasorrah*, in the form of elongated threads, simple and forked

Part II—Scoliodon palasorrah (Cuv)

Scoliodon palasorrah does not occur in such large numbers as *S. sorrakowah*. Twelve pregnant specimens containing 29 embryos, 2 specimens containing eggs in the early blastoderm stage, a pre-pregnant and a post-pregnant specimen, form the material for this study The specimens were collected from November 1936 to ovember 1937.

Reproductive System

The ovaries, as in *S sorrakowah*, are paired structures containing various sized ova and are connected posteriorly with the epigonal organs. The eggs grow to a comparatively large size, about 3 to 4 mm. in diameter. The oviducal opening is common. The ova enter the uteri during the early stages of the process of segmentation The cranial oviduct in a well-developed specimen measures about 50 mm. in length (Photograph 17). The nidamental glands grow to a much larger size than in *S sorrakowah* and are divided into two upper twisted horns and two lower ones. The size of the gland varies according to age, a mature gland measuring 16 mm in length and 8 mm in breadth. Posteriorly the caudal oviduct usually measures about 25 mm

The uterus is divided into as many compartments as there are embryos. The usual number of embryos lodged in each uterus is two, though cases with three have been obtained The embryos grow to a very large size in the uterus of the mother before they are born An embryo measuring 270 mm. with its placenta intact was obtained, showing thereby that they grow to a still larger size. In the early blastoderm stage the egg measures nearly 25 mm. in length and 12 mm in breadth At this stage, the compartments are found to lie almost transversely one below the other (Fig 17). As growth proceeds, they come to occupy a longitudinal position so that two or three compartments are found side by side along the length of the uterus. This shifting in position is due to the unequal growth of the walls of the uterus and of the partitions.

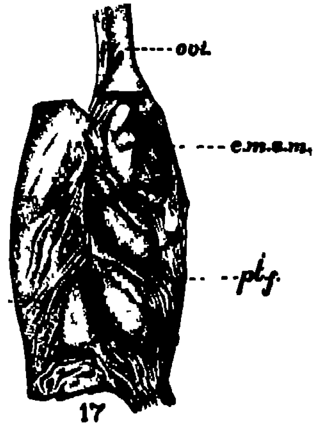
Uterus —The wall of the uterus consists of an outer epithelium, muscular layers—an outer circular, a longitudinal and an inner circular, and a thick layer of sub-mucosa composed of connective tissue and lined by the internal epithelium bounding the uterine lumen.

The changes which occur in the histology of the uterus in the pre-pregnant, pregnant and post-pregnant stages may now be considered.

Pre-Pregnant Uterus

(Photomicrograph 18 and Fig 18)

In this specimen the ovary was well-developed containing ova measuring 3 to 4 mm. in diameter. The walls of the uteri are thick-walled and spongy. The sub-mucosa is thrown into numerous folds, which quite obliterate the lumen The outer epithelial layer is composed of rounded cells with large rounded nuclei filling the entire cell This is followed by a serous layer of connective tissue traversed by a few circular muscle fibres. This, in later stages, forms a well-developed muscle layer Next is a thick layer of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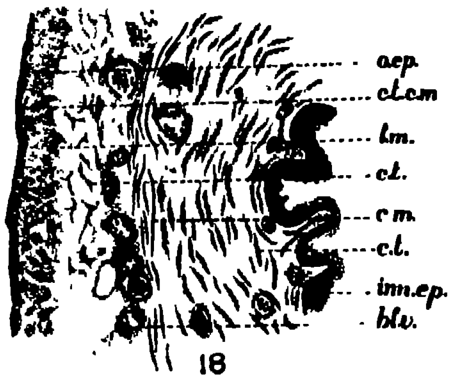
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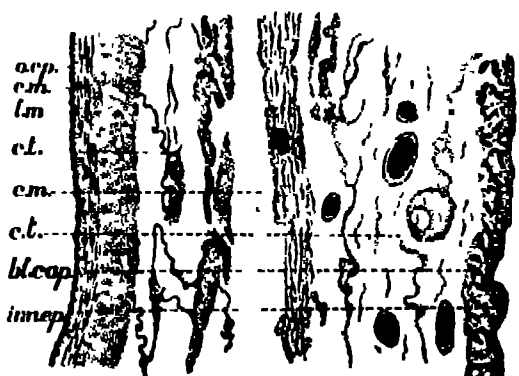
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longitudinal muscles Beneath this is a loose layer of spongy tissue traversed by numerous large blood vessels followed by a layer of well-developed circular muscles. The major part of the sub-mucosa which follows the inner layer of circular muscles is made up of loose well-vascularised connective tissue The inner epithelium is many layered consisting of rounded cells with large ovoidal nuclei.

Early Pregnant Period (Fig 19) —The description here is of an uterus in which an egg in the blastoderm stage was found The structure varies slightly from that of the pre-pregnant uterus. The outer epithelial cells are not quite so rounded and their nuclei which are large and round do not stain very deeply Both the layers of circular muscles are much better developed. There is no marked change in the layer of longitudinal muscles, but the large blood vessels which were present in the region following the longitudinal muscles in the pre-pregnant stage, are now scattered all over the sub-mucosa and not concentrated in that region The connective tissue layer which follows the longitudinal muscles is very loose with numerous spaces in between while the corresponding layer lying internal to the second set of circular muscles is very well developed, compact and well vascularised Here and there numerous blood corpuscles are found to be scattered These groups of corpuscles probably indicate that extravasation takes place The inner epithelium consists of two to three layers of nearly equal cylindrical cells The nuclei are large and more or less basal in position

Late Pregnancy —As pregnancy advances, the uterine wall becomes thinner and transparent so that the embryos containd within could easily be seen The growth is accompanied by enlargement and congestion of the capillaries which at the same time become more numerous As the yolk gets absorbed, the walls of the yolk-sac get folded and in accordance with this the uterine wall also prepares itself to receive this sac preparatory to the establishment of a placenta The uterine epithelium with part of the sub-mucosa raises itself in places and then is invaginated in the form of a cup inside which the epithelium is raised into numerous leaf-like folds supported internally by muscles and connective tissue

The outer epithelial cells which at first are round become more and more flattened The nuclei also stain intensely This layer, due to the extreme stretching of the wall, finally loses its continuity and breaks up here and there (Fig 20) When this happens the cells lose their flattened shape and become short and cylindrical with large nuclei filling up the cells The first set of circular muscles is conspicuous only in the early stages of pregnancy losing its compact nature later and finally gets reduced to a few

loose strands. The longitudinal muscles on the other hand almost throughout pregnancy, remain compact and thick diminishing in thickness only in the latest stages

The loose connective tissue layer following the longitudinal muscles is never very conspicuous The second set of circular muscles is very well developed throughout pregnancy but becomes loose and spread out In between the muscle layers blood vessels occur The region of the sub-mucosa following the second set of circular muscles usually remains compact and well vascularised A glandular sheet-like structure occurs in this region A similar structure occurs in each partition The epithelium which is many layered at first gradually becomes reduced to a single layer with uniformly columnar cells These are coarsely granular and project freely into the lumen.

The nuclei of these columnar cells are terminal in position, though smaller nuclei are present at the base Most of these terminal nuclei are very chromatic As these get cut off the basal nuclei travel up and take their position at the end Several of these cells have been observed lying free in the uterine cavity The cells undergo a process of disintegration and the nuclei along with a little protoplasm get thrown into the lumen. The new epithelium is probably derived mainly from the remaining cells of the old epithelium Whether the epithelium is completely lost cannot be said though in places the sub-mucosa has been noticed to be without a covering epithelium

Between the epithelial layer and basement membrane below numerous capillaries could be seen (Fig 20 *a*). Here also as in *Squatina* and *Heptanchus* the destroyed epithelium affords nutrition The tubular glands which have been mentioned as occurring in the walls of the uterus and the partition secrete a nutritive fluid which goes a long way to nourish the embryos

The general epithelium covering the inner surface of the uterus also takes part in this secretion This fluid when examined was found to consist of numerous, globular, granulated cells of varying sizes with deeply staining nuclei (Photomicrograph 19).

The Post-Pregnant Uterus (Photomicrograph 20 and Figs. 21-22) can easily be ascertained by the presence of the fully formed partition walls and by the remnants of the shell membrane in the lumina of the compartments. The uterus has shrunk greatly and has again become thick walled There is no trace of the trophonematous cups The outer broken epithelium of the final stages of pregnancy has become a continuous layer of small round cells. The cells stain very lightly. The nuclei also are round. Both the circular


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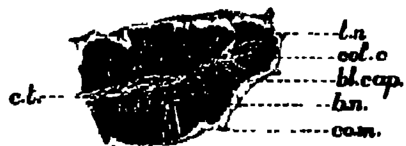
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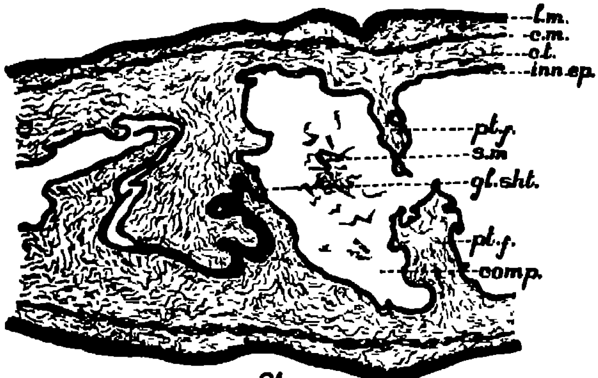
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muscle layers have dwindled much in size but the longitudinal muscles seem to have altered but little. The sub-mucosa is a very loose layer of spongy tissue with just a few strands of muscles here and there. The uterine wall is very poorly vascularised. The inner surface of the uterus is thrown into a few folds which are covered by a layer or two of regenerating epithelium which joins without any sharp distinction the sub-mucosa and the outer border of the epithelium is clearly defined. The cells are short and rounded The charateristic feature of the pregnant stage—the net-work of blood vessels in the sub-epithelial region—is completely absent The glands described during pregnancy in the uterine wall still persist here.

Formation of the Uterine Compartments

As already mentioned two specimens with eggs in the blastoderm stage were obtained Each egg mass was placed in a separate compartment (Fig. 17) The partition walls were thick and ran across the breadth of the uterus On careful examination it was found that the partition was comprised of two overlapping folds, one from the dorsal and the other from the ventral wall of the uterus The partition folds were very well developed. This shows that these might have been formed even before the eggs descended into the uterus in which case the eggs would have slipped in between the loose folds into the compartments Another possibility is that the folds had developed partly before the entry of the eggs into the uterus and when this has taken place, the folds grow rapidly towards each other and overlap, in which case the passage of the eggs would have been along the space between the two partly developed folds In later stages, intimate connection takes place between the two folds This is brought about by the development of glands where the epithelia of the folds come together by the differentiation of the cells (Fig 23) The lumen of the uterus and that of the oviduct get shut off at the anterior end when the compartments take on a longitudinal position This may prevent fresh eggs from descending into the uterus In all the specimens examined, it was found that the uterus contained embryos almost of the same size This shows that during the development of one set of eggs, other eggs are probably prevented from descending into the uterus. At the posterior end the uterus opens into the cloaca The trophonemata are placed at the posterior end of the compartments The surface of fusion of the two partition folds is represented even in the very late stages by a narrow, opaque, thick region running along the centre of the partition sheet (Photograph 21) The folds even at this stage can easily be pulled apart In sections it is found that the epithelia belonging to the two halves of the partition which come together are thrown into zig-zag folds and the folds of one set fit into the depressions of the other set (Photomicrograph 22)

Structure of a Compartmental Wall

(Figs 21 and 23)

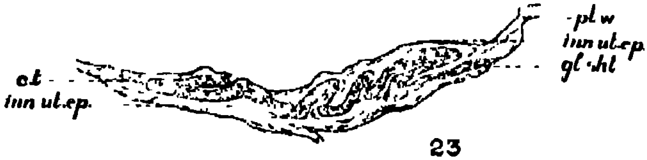
The wall is bounded by the uterine epithelium on either side. In the thickness of the wall runs a layer of connective tissue, traversed by muscle strands The nature of the epithelial cells varies according to the period of pregnancy The glandular sheet which occurs in every partition wall consists of a chain of tubular glands (Photomicrograph 23 and Fig 24). These are numerous, closely placed and cut transversely The cells are non-ciliated, long with round, basal nuclei On either side of these tubular glands a layer or two of cells occur, which are continuous with the inner epithelial cells of the uterine compartments The function of these glands is probably secretory, adding to the secretions of the glandular epithelium.

Placenta

General.—The structure of the placenta could only be understood by tracing its development from an early period of gestation. With the changes in the uterine wall, changes also take place in the yolk-sac of the fœtus

The mature uterine mucosa of the early pregnant uterus already shows specialised structures, the trophonema, which are of importance for the attachment and the nutrition of the embryos Unlike in *S sorrakowah*, the eggs of *S. palasorrah* are big and consequently the yolk-sac of the young embryo is also large This affords nourishment to the embryo for a longer period than in *S sorrakowah* and the early formation of a placenta is therefore unnecessary The yolk-sac is highly vascularised In the early stages when the embryo is just a couple of centimetres long the yolk-sac is a solid bag filled with yolk granules As the embryo grows the yolk gets absorbed and the folding of the yolk-sac commences This folding becomes more and more complicated until finally when all the yolk is absorbed, there remains a much folded and frayed structure, not unlike a cauliflower The folding is more complicated at the distal than at the proximal part of the yolk-sac

Any yolk that is left is confined to the proximal portion of the yolk-sac. The folds of the yolk-sac fit into corresponding depressions of the uterine trophonemata. The entire yolk-sac does not enter the trophonematous cup. Part of the proximal portion of the sac is always free. The trophonematous cup, as previously said, is formed by the raising up of the uterine epithelium along with part of the sub-mucosa and then undergoing invagination The inside of the cup is raised into numerous folds. The formation of the cup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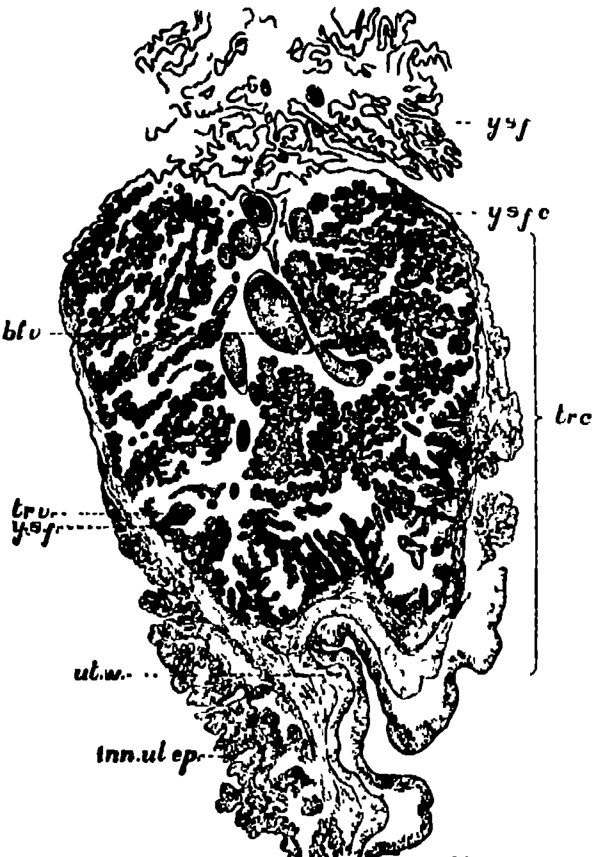
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begins more or less at the same time as the folding of the yolk-sac. The modification of the fœtal tissue extends over the entire surface of the sac, and the folds of the latter continue to advance and line the trophonematous villi. The absorptive surface is increased by the repeated branching of the fœtal folds and the trophonematous villi. Fig 25 shows clearly the very intimate union between these folds

Structure of Yolk-sac before attachment to the Trophonema

The wall of the yolk-sac is made up of three definite layers (Photomicrograph 24) :—

1. There is an outer epithelium, one or more cells deep according to the position The cells are small and round with deeply staining nuclei. The cells at the proximal end of the yolk-sac are columnar, granular, and arranged in several layers with basal nuclei.

2. The second or middle layer is the mesoblast consisting of a layer of flat cells and with a rich supply of blood vessels pervading the sheet of mesoblastic tissue

3 The third layer, the endoderm, is made up of large round cells with rather clear cytoplasm and round nuclei In the region of the yolk-sac, where the two sides of a fold come together, the intervening space is traversed by a number of blood vessels

The lumen of the sac is filled with easily staining yolk granules of different sizes which are perfectly spherical Besides these yolk spherules, there are other round bodies similar to the yolk granules but smaller in size which stain quite differently

In Mallory's stain, the yolk spherules take on a bright, orange-red colour, and the smaller bodies a light blue Coagulated albuminous matter also fills up a good portion of the lumen.

Structure of the Trophonematous Cup before connection with the Yolk-sac

A longitudinal section of this cup at this stage shows numerous villi, each villus (Photomicrograph 25) lined with uterine epithelium and containing internally a vascular core of mesoblast The cells of this epithelium are columnar or rounded according as the villi are situated at the top or the base of the cup. In other words the epithelial cells gradually change from being columnar at the top to being round or almost flat at the base of the cup. Next to this is the mesoblast consisting of a layer of flat cells followed by a very vascular narrow region made up of connective tissue permeated by a net-work of blood vessels. The core of the villus contains musclar strands,

loose connective tissue and a few tiny blood vessels. The folds are simple and not elaborately branched at this stage

Many large blood vessels are concentrated in the uterine wall forming the base of the cup. Tubular glands are also present in this region of the uterine wall The cells of these glands are long with basal nuclei

Structure of the Yolk-sac after Reception into the Trophonematous Cup

The connection between the folds of the yolk-sac and the villi of the trophonematous cup now becomes established The folds of the yolk-sac apply themselves to the surface of the trophonematous villi resulting in an apposition of the fœtal and maternal layers This is the commencement of the formation of the placenta

The yolk-sac is made up of three layers as described above (Photomicrograph 26) The small, round, epithelial cells have now become columnar with deeply staining basal nuclei. Only in some are they terminal The cells are poly-nuclear, the nuclei at the base being smaller Tips of cells project freely and places are noticed where they have been cut off and thrown into the lumen of the uterus The columnar nature of the epithelial cells is lost in the most distal region, where it enters the trophonematous cup The epithelial layer is often found separated from the mesoderm and also from its basement membrane The blood vessels have enlarged and in places have pierced through the endoderm and project freely into the lumen of the yolk-sac The endoderm is thin and consists of irregularly shaped cells with clear cytoplasm The nuclei are round and lightly stained in some and in others flat and deeply stained A few yolk granules yet persist along with albuminous coagulated matter in the lumen of the sac.

At this stage the folds of the yolk-sac do not apply themselves firmly to the trophonematous villi, hence there is a large irregular space between the maternal and fœtal tissue ; besides, there is no regular arrangement of the folds A villus in the trophonematous cup now shows a central core of muscle fibres, enclosed by a continuous net-work of capillaries which, in turn is enclosed by the cells of the uterine epithelium The epithelial cells are now flat and placed rather far apart. Then a large irregular space occurs followed by the three layers of the yolk-sac, the outer epithelium with flattened cells, followed by the vascular mesoderm and lastly by the endoderm layer which now consists of small flattened non-protoplasmic cells The mesoderm layers of the mother and fœtus are very well vascularised, being made up of numerous vessels closely placed. At this stage the maternal and fœtal epithelial layers are very much reduced and consist of a few, very flat cells.

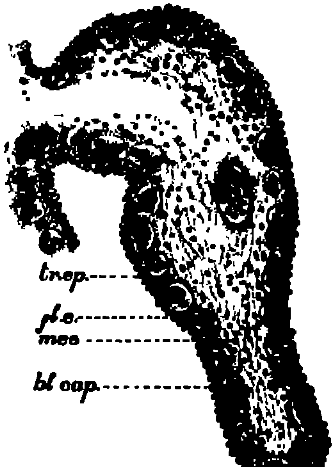
In the space between the maternal and fœtal layer no cement substance is present as in *Mustellus lævis.*

Final Stage of the Placenta

The most advanced stage of placenta studied shows a much folded yolk-sac devoid of any yolk, whose connection with the trophonematous cup is very intimate and firm Even here a part of the yolk-sac is free and does not enter the cup This free part of the yolk-sac in section, now shows a very well-developed active epithelium (Photomicrograph 27 and Fig. 27). The cells are polynuclear and are uniformly long and columnar and granulated, with spherical nuclei The nuclei are mostly terminal and very chromatic. The cells project freely to the outside, and in some, the tips are rounded off and these along with their nuclei, get cut off and thrown into the lumen. The mesoderm does not show much change, but the blood vessels have increased in size and are not quite so numerous The vessels crossing the lumen have increased greatly not only in number but also in size Internally there is a thin endodermal layer

The lumen of the yolk-sac now is devoid of any yolk granules or albuminous coagulated matter but is traversed by numerous blood vessels. In many places, the sac is so much folded that the sides of two folds come together and are connected to each other by connective tissue, blood vessels, etc

The trophonematous cup in this advanced stage is very large and very well developed (Fig 25) The fœtal tissue advances and closely lines the uterine villi, and the arrangement becomes more regular To increase the surface of contact, each fold becomes pinnately branched, the structure therefore becoming more complex (Photomicrograph 28) In between the villi, the spaces are filled with blood vessels, the largest of these occurring in the centre of the cup At this stage a trophonematous villus, together with the enveloping fold of the yolk-sac shows the following changes (Photomicrograph 29 and Fig 28) The core of mesoblast has become very narrow and is traversed by blood vessels and is followed by a very well-developed vascular region. The epithelial layers of both maternal and fœtal tissues consist of very few flat cells with flat nuclei, and the intervening space between the two is extremely narrow, or almost absent. Thus (*a*) an increase and enlargement of the maternal and fœtal capillaries to meet the increasing demands of the growing embryo, (*b*) the attenuation of the uterine epithelium and also of the fœtal yolk-sac epithelium, and (*c*) the pronounced bulging of the maternal and fœtal capillaries over the placental face with their own walls reduced in thickness are the most important changes that take place in the



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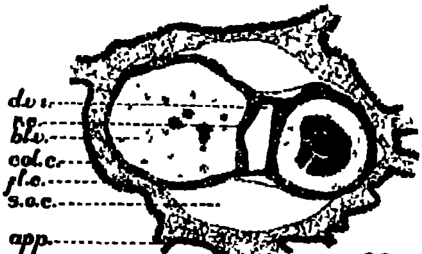

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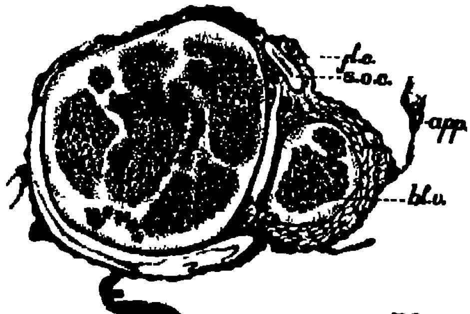

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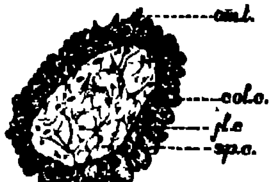

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32

maternal and fœtal tissues so as to bring about a close apposition of the maternal and fœtal blood streams

Placental Cord

In the more highly evolved forms of Elasmobranchs, where a yolk-sac placenta is formed, the channel of the yolk-stalk is obliterated in later stages of development owing to there being no yolk to absorb At this stage an artery and a vein are developed which traverse the length of the cord. Fig 29 is a transverse section of a placental cord. It is seen that the entire core is not taken up by the two blood vessels but in between the two vessels is a central channel, the ductus vitello-intestinalis, bounded by a well-formed layer of distinct rectangular cells with spherical nuclei. Coagulated matrix occurs in the lumen of this channel. On either side of this two other spaces occur All these are the remains of the original yolk-stalk channel

The outer epithelium of the cord consists of rather long highly protoplasmic cells with perfectly rounded nuclei The outer ends of some of these cells are amœboid Next is a layer of flattened cells followed by a spongy mesh-work

In more advanced stages (Fig 30) the blood vessels enlarge, the spaces representing the original channel become fewer and smaller The central channel persists, but becomes narrow and long due to the growth in length and width of the yolk-cord The cells bounding this channel become smaller and flatter which stain very deeply and finally the layer gets broken up due to stretching and in the end disappears.

The columnar cells of the outer epithelium become very conspicuous but lose their amœboid appearance, and still later, this shape is lost and they become smaller, round and less compact and finally the layer breaks up and disappears leaving the layer of flattened cells outermost Blood vessels develop just below this layer, and become larger and more numerous. This rich vascularisation at the periphery is probably to help in absorption

Appendicula

The placental cord gives off numerous appendicula. They are not long as in *S sorrakowah*, but are slender very well branched structures each branch ending in a knob These appendicula are more numerous at the distal than at the proximal end of the cord. In the very advanced stages of pregnancy most of these are destroyed only a few remaining. The histology of the appendicula does not vary much from that of the placental cord, except that in the early stages, the central core of an appendiculum is a solid core of spongy cells (Fig. 31) A layer of flattened cells surrounds this core and the layer of highly protoplasmic columnar cells with

basal nuclei forms the outer rind. The tips of some of these cells are amœboid. The uterine secretion is probably engulfed by the amœboid processes In between the large, columnar cells smaller narrow cells occur In the advanced stages the central core is replaced by two blood vessels At first spaces occur containing corpuscles which later join to form the blood vessels The outer rind of cells lose their columnar shape and their amœboid tips and become short and round Finally, this layer breaks up and disappears leaving the flat cells exposed (Fig 32) Blood capillaries develop at the periphery as in the yolk-stalk The spongy layer completely disappears, muscle strands developing in its stead The rich development of blood vessels on the outer surface is probably to help in absorption of the uterine fluid

Appendix

Two other species of *Scoliodon* which are fairly common on the Madras Coast are *S walbeehmi* and *C dussumieri* A brief description of the placenta of these is given here, as they are referred to, in the discussion that follows

Only two specimens of *S walbeehmi* have been examined both in the pregnant stage measuring 29" and 29 5" respectively One of these was obtained in February 1937 containing three embryos and the other in August 1937 containing two embryos The ovary is well developed in this species with large-sized ova, bigger than those found in *S palasorrah*, and the shell-glands are also better developed

In both the specimens the placenta was developed, and it closely resembled that of *S palasorrah* The distal half of the folded yolk-sac had entered the trophonematous cup and a firm connection between the two established (Photograph 1). In a longitudinal section of the placenta the trophonematous cup was found to be thrown into villi which were lined by the fœtal folds

The yolk-cord is a long thick structure closely beset with appendicula. These are numerous, very short, rather thick and much branched, each branch ending in a knob (Photograph 1) In a transverse section, an appendiculum was found to have the same structure as that of *S sorrakowah* and *S palasorrah.*

Carcharinus dussumieri—Nine pregnant specimens were collected between the months of October 1936 and September 1937, varying in size from 28" to 35·5" Twenty-two embryos were taken from these, varying in size from 80 mm to 390 mm in length

The ova grow to a much larger size, varying from 10–15 mm. in diameter The nidamental glands also are very much larger.

The uterus is divided into as many compartments as there are embryos. The usual number contained in each uterus is two. Each embryo is enveloped in a shell membrane and is bathed in the uterine fluid. The embryos grow to a very large size before they are born The smallest embryos obtained possessed external gill filaments These were simple and measured 13 mm. in length.

Yolk-sac —In the early stages, the yolk-sac in this form is a large bag filled with yolk granules. As the embryo grows, the yolk gets absorbed and the yolk-sac begins to fold from its distal end The lobes given rise to by the folding of the sac, are filled with a clear reddish fluid This fluid soon gets absorbed and the folds become frayed out The structure forms a fairly large arborescent mass In the final stages it acquires a greenish tinge and the outer surface becomes smooth and even due to the continued subdivision of the folds. The proximal-most region gets only slightly folded

During these stages of the growth of the yolk-sac, the inner uterine wall at the posterior end of the uterus also undergoes a slight change The surface becomes rough and highly vascular and the modified yolk-sac merely rests on this region There is no definite placenta formed as in the other forms described. This is due to the well-filled yolk-sac which supplies nourishment to the growing embryos for a considerable length of time. A regular connection with the mother has therefore become apparently unnecessary.

The yolk-stalk is a long thick smooth structure, and the appendicula, characteristic of other forms are completely absent here.

Discussion

Placenta —Southwell and Prashad distinguish three distinct grades in the development of the placenta : (1) The least modified type occurs in *S. sorrakowah* and *S palasorrah*. Here the lower free extremity of the yolk-sac has a number of small protuberances which get embedded in the maternal uterine tissue and form a very simple type of yolk-sac placenta. (2) A more advanced type is found in *Mustelus lævis* (mentioned and described by Muller) Here there is a distinct placenta-like interdigitation of folds of the yolk-sac, and these villi fit into corresponding depressions in the uterine mucous membrane of the mother, like the cotyledons of the ruminant placenta. (3) In the third, the most advanced, the yolk-sac disappears as such, and the placental cord broadens out into a flattened structure showing traces of division and transformation into an arborescent mass This type of placenta was found in a species of *Scoliodon* from Ceylon. An intermediate type between this and the second type of placenta is that found in *C dussumieri*

and *S. walbeehmi* Here a fairly large arborescent structure is formed by the continued sub-division of the distal extremity of the placental cord and the remains of the yolk-sac. This is in close connection with a flat highly vascular portion of the maternal uterine wall From the above statements it is clear that they consider the placenta of *S sorrakowah* and *S palasorrah* as the least modified and that of *S walbeehmi* and *C dussumieri* as the most advanced type, barring the type found in a species of *Scoliodon* from Ceylon. From the study of the placenta made by me of these forms I am led to a different conclusion. I find that the placenta of *C dussumieri* is the most primitive, the placenta of *S sorrakowah* the most advanced and the placenta of *S. palasorrah* and *S walbeehmi* to be intermediate in character between that of *S sorrakowah* and *C dussumieri*

In *S. sorrakowah* it has been noticed that the yolk-sac gets connected with the trophonemata at a very early stage It has been pointed out that both the yolk-sac and part of the trophonemata with which it is connected undergo important changes resulting in an almost complete union between the two structures To describe such a structure as belonging to the simplest type of placenta can only be explained by the fact that the authors seem to have been unaware of the development of the trophonemata The trophonema in this form is a very conspicuous structure especially in early pregnancy when the embryo measures about 20 mm At this stage the trophonema is longer and very much broader than the embryo itself and occupies almost the entire lumen of the compartment Though they get reduced in size in the advanced stages, they continue to be fairly prominent and are placed one behind the other at the posterior end of the uterus They get absorbed in the very final stages.

The placenta of *S palasorrah* which they consider the least modified closely resembles the placenta of *Mustelus lævis* described by Hoedemaker In both, the maternal placenta is built of strongly branched outpushings of the uterine wall, the villi, which are covered by the embryonal tissue. Southwell and Prashad were apparently unaware of the complicated arrangement of the folds of the yolk-sac and the trophonematous villi in this form and this probably explains why they regarded the structure as being primitive.

They also consider the placenta of *S walbeehmi* and *C dussumieri* to be very similar to each other and to be of the same type This is not borne out by my observations. In neither of them does the end of the placental cord sub-divide as stated by them *S walbeehmi*, judged by its placental structure, should be placed under the same group as *S palasorrah* and

Mustelus lævis. There is a trophonematous cup with villi which are covered by the folds of the distal end of the modified yolk-sac.

C. dussumieri builds no definite placenta at all The large arborescent structure formed by the continued sub-division of the distal extremity of the yolk-sac merely rests on the highly vascular portion of the material uterine wall. There is no interlocking of the villi and folds as in *S palasorrah* or *S walbeehmi*. I consider this the most primitive type of placenta. The maternal tissue here hardly undergoes any modification. But according to Wood Mason and Alcock (1890) " each fœtus has its own placenta " They do not describe the placenta

In *Mustelus lævis* Hoedemaker mentions the occurrence of a non-cellular homogeneous membrane between the maternal and fœtal tissues of the placenta. This membrane, which is the shell membrane, does not occur in the placenta of *S palasorrah* Probably it gets broken up or absorbed in that region where the embryonal tissue comes in contact with the maternal tissue He also states that a kind of cement substance connects the shell membrane, with the uterine epithelium of the paraplacental region No such substance has been noticed in *S palasorrah*

Appendicula.—Southwell, Prashad and Tillayampalam state that in species with the best developed appendicula the placenta is of the most primitive and least evolved type and *vice versa*, and " Indeed, this last stated fact seems to show that the forms with a less highly organised type of placenta requiring some other mode of absorption of food have developed these additional structures". This statement of theirs does not tally with the observations made by me in the forms of *Scoliodon* *C dussumieri* builds no placenta and yet the placental cord is completely devoid of appendicula. I find the best developed appendicula in forms with the best developed placenta. In *S sorrakowah*, where the yolk-sac is of the size of a pin-head with hardly any yolk, and which develops a placenta at a very early stage has the longest and thickest appendicula, though not much branched Next comes *S palasorrah* where the eggs are rather big but not so big as those of *S walbeehmi* or *C. dussumieri* In this the yolk-sac is larger than that of *S. sorrakowah* and the placenta is developed when the embryos are rather big Here the appendicula are not so well developed as in *S. sorrakowah* being thinner and shorter but are well branched. Next comes *S walbeehmi* The eggs in this form are very large, hence yolk-sac also must be big with plenty of yolk. Here the appendicula are very short and branched. Lastly, *C. dussumieri* has the largest yolk-sac among the forms studied and a regular placenta is

never developed. This form is devoid of appendicula These points go to show that S. ***sorrakowah*** with the smallest egg has the best developed placenta and is the most highly evolved, following which comes S ***palasorrah*** and S. ***walbeehmi*** The most primitive of the four is C ***dussumieri*** with the largest egg, no placenta and no appendicula.

In conclusion, from the observations made, it is found that the placenta of S. ***sorrakowah*** and S. ***palasorrah*** are fundamentally similar but have differences in structure which immediately suggest that one (***palasorrah***) is not so highly specialised as the other (***sorrakowah***) In S ***sorrakowah***, the structure of the placenta attains its highest stage of progressive histological differentiation. The yolk-sac is a spongy ball saturated with circulating blood. It grows in size as the embryo grows to fulfil more adequately the function of transferring nourishment from the mother to the fœtus The uterine epithelium in the trophonematous cup becomes highly specialised The cells become columnar and the relationship with the yolk-sac epithelium becomes very intimate, much more intimate than in S ***palasorrah*** S. ***walbeehmi*** and C ***dussumieri*** should be regarded as less specialised than either S ***sorrakowah*** or S ***palasorrah***

Summary

Part I

1. The material for this study consists of uteri of thirteen specimens, all in the pregnant stage.

2 Each uterus is divided into as many compartments as there are embryos. The partitions show glandular structures which are described for the first time

3. Though viviparous, each embryo is enveloped in a delicate shell membrane

4 At the base of each compartment there is trophonema, a special process of the uterine mucosa described in detail in this species for the first time

5 The mature egg is very small and the mother is driven to the necessity of making early provision for the growth of the embryo This explains the quick formation of a placental connection

6 The yolk-sac placenta is formed by the modified spongy yolk-sac getting embedded in the trophonematous cup The structure of the placenta is described.

7. In the placenta, though fœtal tissue is attached to the maternal tissue, there is no invasion of the maternal tissue by the fœtal tissue.

8. The placental cord is beset with numerous long thread-like appendicula. These are simple and rarely branched.

Part II

1 Twelve pregnant specimens containing twenty-nine embryos, two specimens in the early blastoderm stage of pregnancy, a pre-pregnant and a post-pregnant uterus formed the material for the study.

2. The egg when it descends into the uterus is enveloped in a shell membrane

3 The egg is big, and a large yolk-sac is formed with well-filled yolk.

4 The uterus is divided into as many compartments as there are embryos.

5 The partition wall is comprised of two folds, one from the dorsal and the other from the ventral wall of the uterus These overlap and intimate connection between the two takes place later.

6. A glandular sheet occurs in every partition wall consisting of a chain of tubular glands.

7 At the posterior end of each compartment a trophonematous cup is formed by the raising up of the uterine mucosa and by invagination. The inside of the cup is thrown into numerous villi

8 As the yolk gets absorbed the yolk-sac begins to fold from its distal end This folded portion of the yolk-sac enters the trophonematous cup resulting in a close apposition of the fœtal and maternal layers

9 There is a much greater surface of contact between the maternal and fœtal tissue in *S palasorrah* than in *S sorrakowah*

10. The core of the placental cord is occupied by two blood vessels between which is a central channel, the ductus vitello-intestinalis which is the remains of the original channel of the yolk-stalk.

11 The appendicula are short and much branched.

12 It is pointed out that the placenta of *Scoliodon sorrakowah* is the most specialised and that of *Carcharinus dussumieri* the most primitive of the forms dealt with in this paper

Acknowledgment

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* Have not been referred to in the original. The list also contains a few references not actually mentioned in the text.

EXPLANATION OF FIGURES

Scoliodon sorrakowah

FIG. 1.—Posterior region of the uterus. Advanced pregnancy, showing the yolk-sac placenta, the trophonema and the partition folds. × 1½

FIG. 2.—The reproductive system (diagrammatic).

FIG. 3.—T.S. of early pregnant uterus, showing the compartments containing the embryos and the glandular sheet in the partition walls. × 14.

FIG. 4.—Early pregnant uterus, showing the compartments, embryos, yolk-sac placenta and trophonema (diagrammatic).

FIG. 5.—T.S. of the wall of an early pregnant uterus. × 40.

FIG. 6.—T.S. of an uterine fold. Early pregnancy, showing the inner epithelium and the net-work of blood vessels in the sub-epithelium. × 400.

FIG. 7.—T.S. of the wall of an advanced pregnant uterus. × 280.

FIG. 8.—T.S. of the partition wall, showing the glandular sheet. × 200

FIG. 9.—L.S. of the yolk-sac placenta. Early pregnancy, showing the yolk-sac embedded in the trophonematous cup. × 40.

FIG. 10.—L.S. of a fold of the trophonematous wall. Early pregnancy. × 200

FIG. 11.—L.S. of a fold of the trophonematous wall. Advanced pregnancy. × 280.

FIG. 12.—L.S. of the wall of the trophonematous cup and yolk-sac Early pregnancy × 280.

FIG. 13.—L.S. of the wall of the trophonematous cup and yolk-sac Advanced pregnancy. × 280.

FIG. 14.—T.S. of an appendiculum. Early pregnancy. × 400.

FIG. 15.—T.S. of an appendiculum. Advanced pregnancy. × 400.

FIG. 16 —T.S. of an appendiculum. Late pregnancy. × 200.

Scoliodon palasorrah

FIG. 17.—Uterus slit open, showing the egg masses one in each compartment, and the partition folds (N.S.).

FIG. 18.—T.S. of the wall of the pre-pregnant uterus. × 120.

FIG. 19.—T.S. of the wall of the early pregnant uterus. × 80.

FIG. 20.—T.S. of the wall of the late pregnant uterus × 40.

FIG. 20*a*.—T.S. of a fold of the inner epithelium to show the columnar cells. × 160.

FIG. 21.—T.S. of a post-pregnant uterus. × 20.

FIG. 22.—T.S. of the wall of the post-pregnant uterus. × 80.

FIG. 23.—T.S. of the partition wall, showing the glandular sheet × 56.

FIG. 24.—T.S. of the glandular sheet, showing the tubular glands with basal nuclei. × 200.

FIG. 25.—L.S. of the placenta. Advanced stage, showing the proximal half of the folded yolk-sac, the trophonematous cup and the trophonematous villi covered by the yolk-sac folds. × 10.

FIG. 26. L.S. of a trophonematous villus before the entry of the yolk-sac folds into the cup. × 200.

FIG. 27.—L.S. of part of the yolk-sac, showing a fold lined with columnar cells. × 200.

FIG. 28.—L.S. of a trophonematous villus covered by the yolk-sac folds. × 280.

FIG. 29.—T.S. of placental cord. Early stage, showing the ductus vitello-intestinalis. × 56.

FIG. 30.—T.S. of placental cord. Advanced stage. × 56.

FIG. 31.—T.S. of an appendiculum. Early stage. × 280.

FIG. 32.—T.S. of an appendiculum. Advanced stage. × 280.

N.B.—The magnifications given for the Text-Figures are of the original drawings. They have all been reduced to half their size.

EXPLANATION OF PHOTOGRAPHS

PHOTOGRAPH 1.—Uterus of *S. walbeehmi* slit open, showing the embryos, placental cord, appendicula and yolk-sac placenta.

PHOTOGRAPH 2.—Embryo of *S. palasorrah* with its placental cord, appendicula and yolk-sac placenta.

PHOTOGRAPH 3.—Embryos of *S. sorrakowah* with their placental cord, appendicula and spongy yolk-sac.

PHOTOGRAPH 4. Embryo of *Carcharinus dussumieri* with its placental cord and the folded yolk-sac, the proximal region of the sac containing fluid.

PHOTOMICROGRAPH 5.—T S. of an early pregnant uterus, showing the compartments with embryos and the glandular structures in the uterine and partition walls.

PHOTOMICROGRAPH 6.—T.S. through the posterior region of a compartment, showing the trophonemata.

PHOTOMICROGRAPH 7.—An embryo of *S. sorrakowah* (measuring 20 mm.) with its placental cord and appendicula

PHOTOMICROGRAPH 8.—T S. of part of a compartment, showing embryo cut, appendicula, partition wall, glandular structures in the partition, and the extremely vascular sub-epithelium.

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PHOTOMICROGRAPH 29.—L.S. of part of a villus lined with fœtal tissue. Highly magnified.

KEY TO LETTERING

am.t	Amœboid tips.
app.	Appendicula.
b.n.	Basal nucleus.
bl.cap.	Blood capillaries.
bl.v.	Blood vessel.
c.bl.v.	Chain of blood vessels.
cl.	Cloaca.
c.m.	Circular muscle layer
c m.f ct.	Core of muscle fibres and connective tissue.
col.c.	Columnar cells.
comp.	Compartment.
c.t.	Connective tissue.
co.m.	Coagulated matrix.
c.t.c.	Connective tissue core.
c.t.c.m.	Connective tissue with a few circular muscle fibres.
d v.i	Ductus vitello-intestinalis.
emb	Embryo.
e.m.s.m.	Egg mass in the blastoderm stage enveloped in a shell-membrane
f p.	Finger-shaped process.
fl.c.	Flat cells.
gl.c.gr.	Gland-cell groups.
gl.ep	Glandular epithelium
gl.sht.	Glandular sheet
inn.ep.	Inner epethelium
in.ut ep	Invaginated uterine epithelium
inn ut.ep.	Inner uterine epithelium
l m.	Longitudinal muscle layer
mes.	Mesoblast
nid.gl.	Nidamental gland.
o.ep	Outer epithelium.
ov.	Ovary.
ov.f.	Oviducal funnel.
ovi.	Oviduct.
pl.c.	Placental cord.
pt f.	Partition folds.
pt.w.	Partition wall.
r.c.	Rectangular cells.
s.o.c.	Spaces of the original yolk-stalk channel.
s.m.	Shell membrane.
sp c.	Spongy core.
sp n.	Spongy net-work.
t g	Tubular glands.
t n.	Terminal nucleus.
tr.	Trophonemata.
tr.c.	Trophonematous cup.
tr.ep	Trophonematous epithelium.
tr v.	Trophonematous villus.
ut	Uterus.
ut ep	Uterine epithelium.
ut.w	Uterine wall
vas peri reg.	Vascular peripheral region
w y.s.	Wall of yolk-sac.
w.tr c.	Wall of trophonematous cup.
y.ect.	Yolk-sac ectoderm
y ect col c	Yolk-sac ectoderm of columnar cells.
y end.	Yolk-sac endoderm
y g	Yolk granules.
y mes.	Yolk-sac mesoderm
y.s	Yolk-sac.
y.s.f.	Yolk-sac folds.
y s.f.c	Yolk-sac folds on the outer rim of the cup before they turn inward to enter the cup.

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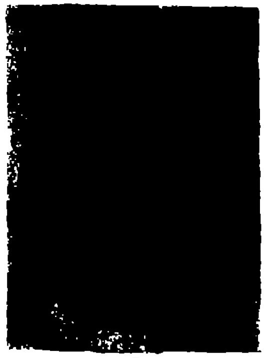

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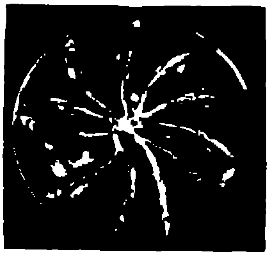

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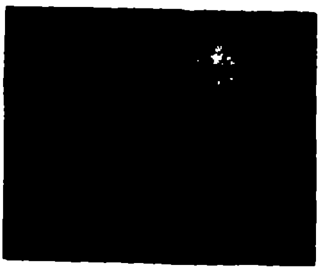

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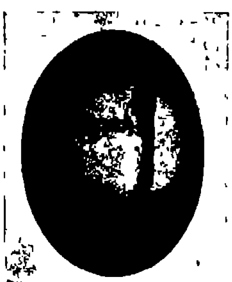

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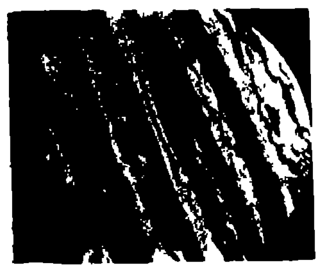

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A NEW SPECIES OF *BOMBARDIA* (*B. HYALINA* SP. NOV.) OCCURRING ON DRY TWIGS OF *THUNBERGIA GRANDIFLORA* ROXB.*

By G S Verma, M Sc

(*Research Fellow, University of Lucknow*)

Received November 6, 1939

(Communicated by Dr S. N. Das Gupta)

A few dry twigs of *Thunbergia grandiflora* infected with a fungus were collected by Dr S N Das Gupta at his Lucknow residence in September 1936 Similar collections were made by the author in succeeding rainy seasons The fungus was never found to infect healthy green plants and was invariably noticed to occur on dead twigs as a saprophyte under conditions of excessive moisture

Description of the fungus—From an examination of the material it was seen that the fungus is an ascomycete producing numerous perithecia well visible with the naked eye (Plate V, Fig 1) The perithecia are superficial, scattered or aggregated in loose clusters, ovate, carbonaceous, measuring 400–500 μ and immersed in a thick weft of mycelium with a prominent ostiole at the end (Plate V, Fig 2) The asci are cylindrical, measuring 150–175 $\mu \times 15 \mu$ with eight spores without any guttalæ (Plate V, Figs. 3 *a* and 3 *b*). The ascospores while in the asci or when just discharged are hyaline, vermiform, 1-celled, contain oil drops and measure 50–65 μ × 6–7μ (Plate V, Fig 4 *b*) The spores undergo peculiar structural development soon after their discharge, becoming multicellular such that in mature condition they consist of an egg-shaped head, a cylindrical tail and a colourless appendage at each end (Plate V, Fig 4 *b*) The spores get thickened at one end to a width of 10–12 μ, the head so formed is generally 2-celled and hyaline, rarely remaining unicellular (Plate V, Figs 4 *c* and 4 *d*) The tail also develops septa showing 4- to 7-celled structure and measures 45–50 $\mu \times$ 6–7 μ (Plate V, Figs 4 *c*, 4 *f*) The head appendage in some cases showed septation (Plate V, Figs 4 *g*, 4 *h*).

The fungus under description has spores which agree in all respects with the spores of *Bombardia* excepting that they are hyaline *Bombardia*,

* This paper was read at the 26th Session of the Indian Science Congress (Lahore, 1939).

however, belongs to the group Phæosporæ and should necessarily have dark spores. Dr G. R. Bisby of the Imperial Mycological Institute, Kew, in a private letter to the author expresses the view that it would unnecessarily increase the genera to make a new genus for a fungus showing all the characters of *Bombardia*, but differing only in spore colour and the author is inclined to agree with him. Further, Cain (1934) has already included in the genus *Bombardia* a species having hyaline spores of *Bombardia* type (*Bombardia muskokensis*) *Bombardia* was erected by Fries (1849) to include fungi of such description growing on wood When growing on dung, fungi of this type were called *Podospora* until in 1911 Kirschstein combined these two genera under *Bombardia* Cain (1934) in his monograph on coprophilous Sphæriales follows Kirschstein About twenty species are known on dung or decaying plant parts in temperate regions but very few species are recorded from tropical or subtropical areas

The species described here differs from all other species of *Bombardia* in specific character. It shows some resemblance to *Bombardia ambigua* Sacc. but differs from the latter in the form of perithecia and in spore character, particularly in the possession of appendages at both ends of the spores The author, therefore, proposes a separate specific name for the fungus and terms it *Bombardia hyalina* sp nov

Culture characters.—An effort was made to procure as many species of *Bombardia* as possible in order to compare their culture characters But only one authentic species, namely *Bombardia setosa*, was available from Baarn, Holland. These two species were therefore utilised for a comparative study

The two species, *Bombardia setosa* and *Bombardia hyalina* sp. nov, were cultured in Agar, Brown's, Coon's, Richard's, Barnes', Malt agar, Potato agar, and Thunbergia extract agar media The characters of these two fungi as developed in these media were noted The results are given in Tables I and II.

A comparison of the two tables shows that the mycelium of *Bombardia setosa* is grey in colour, whereas that of *Bombardia hyalina* is whitish grey. In culture, *Bombardia setosa* shows definite zonation but no zonation appears in *Bombardia hyalina* (Plate VI, Figs 8, 9) The substratal coloration, however, differs with the medium employed For example, *Bombardia hyalina* has transparent substratum in all media except Potato agar and Thunbergia extract agar, where colour is dark In the case of *Bombardia setosa*, the substratum is transparent in the case of agar, Coon's, Barnes' and Thunbergia extract agar, and dark in the rest.

TABLE I

The Characters of Bombardia hyalina *as developed in Various Media*

Name of medium	Perithecial formation	Substratum	Colour of hyphæ
Agar 2 %	nil	transparent	whitish grey
Brown's	,,	,,	,,
Coon's	,,	,,	,,
Richard's	,,	,,	,,
Barnes'	,,	,,	,,
Malt agar	,,	,,	,,
Potato agar	,,	dark	,,
Thunbergia extract agar . ..	,,	,,	,,

TABLE II

The Characters of Bombardia setosa *as developed in Various Media*

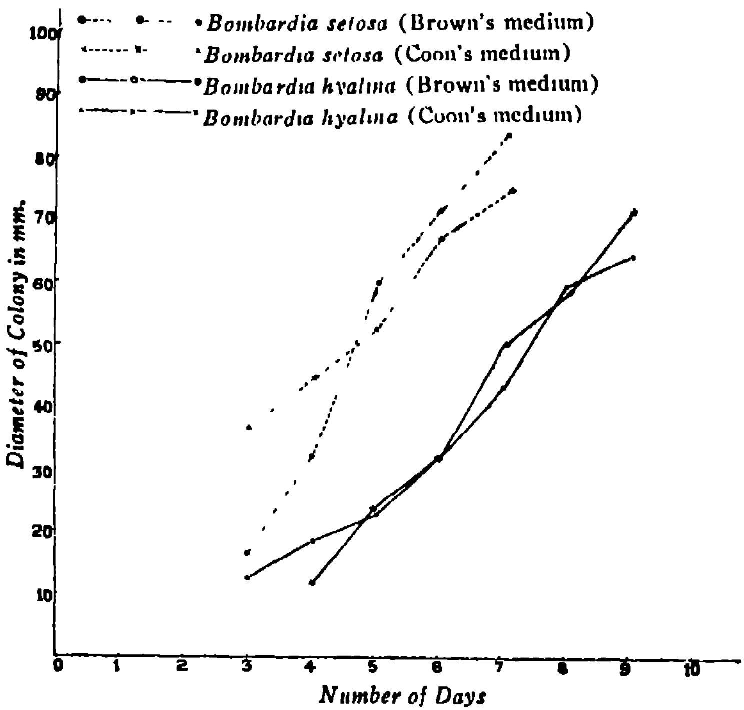
Name of Medium	Perithecial formation	Substratum	Colour of hyphæ
Agar 2 %	few	transparent	grey
Brown's	profuse	dark	,,
Coon's	,,	transparent	,,
Richard's	nil	slightly dark	,,
Barnes'	,,	transparent	,,
Malt agar	profuse	dark	,,
Potato agar	moderate	,,	,,
Thunbergia extract agar	nil	transparent	,,

Bombardia hyalina does not produce perithecia in any of the synthetic media employed, while *Bombardia setosa* produces perithecia in all media except in Richard's, Barnes' and Thunbergia extract agar As most luxuriant growth of perithecia of *Bombardia setosa* was found in malt agar medium, the perithecial and spore characters as developed in this medium were noted and compared with those of *Bombardia hyalina* found on the dead twigs of *Thunbergia grandiflora* Roxb The perithecia are carbonaceous, elongate, and measure 800–900 μ × 500–50 μ (Plate VI, Fig. 5). The asci measuring

200–250 $\mu \times$ 20–24 μ contain an indefinite number of spores (Plate VI, Fig. 6). The spores measuring 10–20 $\mu \times$ 10–12 μ with an appendage measuring 10–12 μ are dark at maturity (Plate VI, Figs 7, 10) Thread-like paraphyses are also seen.

Both perithecia and asci are larger in *Bombardia setosa*, but the spores and appendages are definitely smaller in size

The growth-rate of the two fungi was studied in Brown's and Coon's media and the results are represented in the following graph (Text-Fig 1).



TEXT-FIG. 1

It will be seen from the graph (Text-Fig 1) that *Bombardia setosa* is definitely faster both in Coon's and Brown's synthetic media

Summary

A new species of *Bombardia* obtained from dry twigs of *Thunbergia grandiflora* Roxb. is described. The spores are hyaline and in mature condition consist of an oval head, a tail and two appendages, all of which are usually septate. The fungus is named *Bombardia hyalina* sp. nov

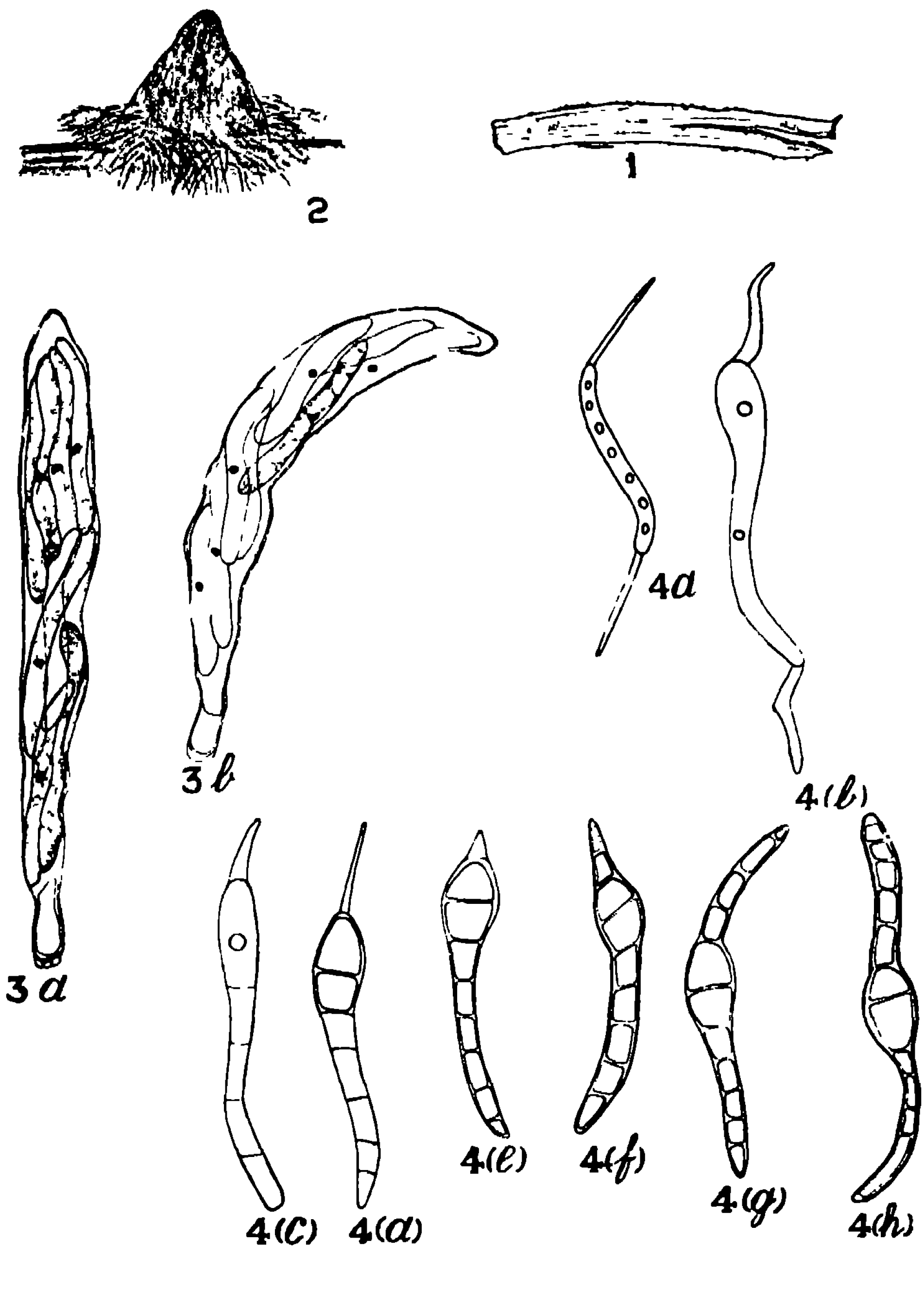

2
1
3 a
3 b
4a
4(b)
4(c)
4(d)
4(e)
4(f)
4(g)
4(h)

Fig. 8

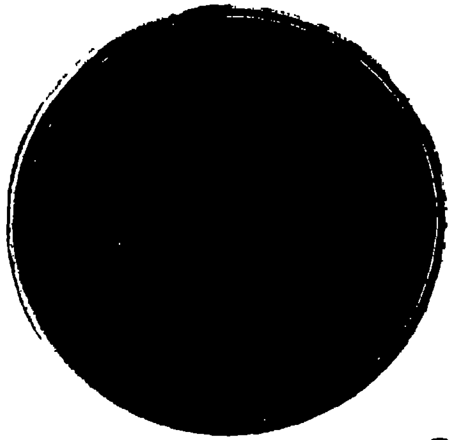


Fig. 9

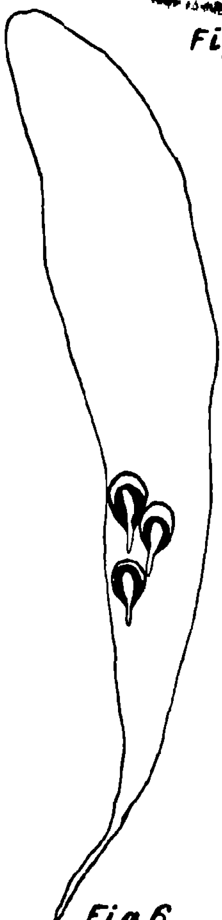


Fig. 6

Fig. 10

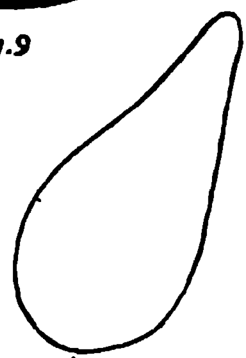


Fig. 5

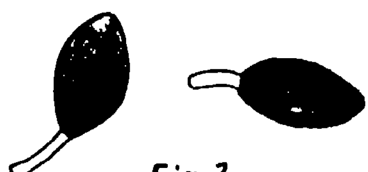


Fig. 7

This species is compared with *Bombardia setosa*, the only other authentic species available

The author wishes to record his grateful thanks to Dr S N. Das Gupta, Ph D (Lond), D I C., and Dr. G. R. Bisby for their kind help.

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EXPLANATION OF PLATES

PLATE V

(*Bombardia hyalina*)

FIG. 1.—A twig bearing perithecia. × 2/3.

FIG. 2.—Perithecium. × 106

FIG. 3.—(*a*, *b*) An ascus with ascospores. × 130.

FIG. 4.—(*a*, *b*, *c*, *d*, *e*, *f*). Ascospores showing different stages of maturity. × 653.

FIG. 4.—(*g*, *h*) Ascospores showing septate head appendage. × 653.

PLATE VI

FIG. 5.—A perithecium of *Bombardia setosa* × 61.

FIG. 6.—An ascus with young spores of *Bombardia setosa* × 610.

FIG. 7.—Two mature spores. × 892.

FIG. 8.—Microphotograph of a culture of *Bombardia hyalina*, showing complete absence of perithecia in Brown's synthetic medium × ·68.

FIG. 9.—Microphotograph of a culture of *Bombardia setosa* showing zonation and profuse perithecial formation in Brown's synthetic medium × ·68.

FIG. 10.—A microphotograph of mature asci of *Bombardia setosa* × 119

ON *HERDMANIA (RHABDOCYNTHIA) ENNURENSIS* N.SP. (A NEW MONASCIDIAN FROM MADRAS)

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(Communicated by Prof. A. Subba Rau)

CONTENTS

I Introduction

The present contribution is the second of a series[1] (1938) in which the author desires to add to the existing knowledge of the ascidian fauna of the Indian coasts. Except for Herdman's work[5] (1906) very little systematic work on ascidians has been done in India Oka[8] (1915) published a report on the ascidians in the collection of the Indian Museum, Calcutta ; but the marjoity of the species reported were those already described by Herdman[5] (1906) or by other workers in the West. The only other publications on Indian Tunicata are by myself,[2, 3] (1936) on the morphology of *Herdmania*

pallida; by Lall[7] (1938) on some Tunicates from Karachi, and by Sebastian[8] (1939) on the development of *Herdmania pallida.*

The external form, shape and coloration of preserved ascidians are often misleading on account of contraction and post-preservation changes I propose, therefore, to lay more stress on internal characters as criteria for specific distinction

The material was received in a preserved condition from the Marine Biological Supply Station at Ennur (Madras) There is no label or report showing the place and time of collection Presumably the specimens were collected either from the Madras coastal region or from the coast at Tuticorin (S India), where animal supplies for the Biological Station at Ennur are collected Only three specimens of the present species were received, but they were in a well preserved state for necessary examination

My acknowledgements are due to the management of the Ennur Station for supplying me the material To Prof. K N Bahl of Lucknow I am deeply indebted for obtaining the material and for finally revising the manuscript Thanks are due to Prof Huus of Oslo and Dr Van Name of the American Museum of Natural History for personally communicating to me their views on the validity of the genus *Herdmania.*

II Historical

The genus *Rhabdocynthia* was first instituted by Herdman[6] (1891) to include those species of *Cynthia* (now *Pyura*) that possess calcareous echinated spicules in the body-tissues In 1906[5] while advocating the desirability of retaining the genus *Rhabdocynthia*, originally established by him, Herdman wrote " Notwithstanding Michaelsen's remark (1905) and the fact that several recent writers have seen fit to relinquish the genus *Rhabdocynthia*, I believe it is both natural and useful to group together those species of *Cynthia* that show echinated unbranched calcareous rods or spicules in the connective tissue of the body The grouping of species into genera is largely a matter of convenience, and if a set of closely related species can be defined and recognized by the possession of a common character, the application of a generic name seems justifiable and is certainly an aid in classification On these grounds I make use of *Rhabdocynthia* as the generic designation of the set of species which may be grouped around Heller's '*Cynthia pallida*' "

In the same paper, however, Herdman makes a note that if this is exactly the genus for which Lahille (1888) proposed the name *Herdmania*, then *Rhabdocynthia* must give way to that prior designation Later

Hartmeyer[4] (1910) established the identity of *Rhabdocynthia* Herdman and *Herdmania* Lahille, and pointed out that on anatomical characters these two genera should be merged in the common genus *Pyura* (*Cynthia*), and that the presence of spicules was not a very distinguishing character In spite of Hartmeyer's statement and Michaelsen's agreeing with his view, I accepted the genus *Herdmania*[2] (1936) as I found no record of an ascidian other than a Pyurid (and that also in closely related species) which showed echinated, unbranched calcareous spicules (knob-shaped, spindle-shaped or pipette-shaped) in the test and connective tissue of the body And if certain Pyurids had this character (*i e*, the presence of echinated calcareous spicules in the test as well as in the connective tissue) in common, I thought it useful to retain the genus *Herdmania* for them

Johan Huus, author of 'Tunicata' in Kukenthal Series, wrote to me in 1937 "As to the genus *Rhabdocynthia* or *Herdmania*, I do not find the presence of rod-like spicules in the tissues being reason enough for maintaining it as a valid genus I quite agree with Hartmeyer[3] (1910) and Michaelsen that it should be treated as a synonym of *Pyura* It should be remembered that not rod-like spicules (stellate, dumb-bell, etc) are also found in several Pyurid species, *e g*, *P pachydermata*, *P gibbosa*, *P australis* Moreover in *P bradleyi* Van Name, stellate spicules occur along with rod-like forms (Van Name,[10] 1931) These facts are obvious against the maintenance of the genus *Herdmania* as defined by Herdman and now by you" Despite this note by Huus I do not think the case strong enough to relinquish the genus *Herdmania* for the broader genus of monascidians, *Pyura*, which comprises a vast number of species The fact that Van Name[10] (1931) has discovered the widely found stellate spicules along with rod-like ones in *Pyura bradleyi* does not depreciate my argument If the rod-like spicules are found in the test and connective tissue and if they are echinated, *Pyura bradleyi* should be renamed *Herdmania bradleyi* But then the spicules found in *P bradleyi* by Van Name, though rod-shaped, are not echinated like those of *Herdmania*, and this discovery, therefore, does not stand against my maintenance of the genus *Herdmania* *The facts that* (i) *echinated calcareous spicules are not found in all Pyurids*, (ii) *that they are found only in closely allied species*, and (iii) *that wherever they are present they are abundant and conspicuous, induces me to maintain that their presence should constitute a character of generic importance* Van Name (April 1939) also comes to the same conclusion when he writes "*Pyura bradleyi* from the west coast of South America has some needle-like spicules in the walls of the blood vessels but they bear little resemblance to those found in *Herdmania* which I regard as a good genus, ... ".

III. External Characters

The animal (photograph) is pyriform in shape and small in size, the largest individual measuring 22 mm in length (the length being the distance from the attached end to the base of the siphons) and 20 mm broad The attached end consists of a narrow base while the body is broadest at its middle. The coloration in the preserved specimens is dull white, although this is no clue to the actual colour of the living forms The siphons are the most prominent structures visible externally, both the siphons being elongated and much longer than in the three species of *Herdmania* already described from the Indian coasts The atrial siphon in the present species is longer than the branchial being almost half the length of the body The maximum measurement for the atrial siphon was 12 mm and that for the branchial 7 mm The siphons are about 5 mm apart at their bases, while the siphonal apertures are approximately 16 mm apart This is due to the fact that the siphons diverge from each other as they arise from their bases and extend more or less upwards and outwards The atrial aperture is directed sideways while the branchial remains nearly upright Each siphon is about 7 mm broad at the base, and tapers towards the distal extremity The siphonal apertures are bounded by four lips of the test, while the extension of the test, lining the interior of each siphon, has four longitudinal double rows of white-spotted streaks clearly seen in the photograph

All the three specimens had, what appeared at first sight, stellate hair, forming a fine down all over the surface of the test Even under a binocular microscope they appeared very much like the hair (projections of the test) described and figured by Herdman[6] (1906) for *Cynthia* (*Pyura*) *crinitistellata* from the Gulf of Manaar But a close examination revealed them to be extensive hydroid colonies of *Bougainvillia* sp This is significant as it shows how mere external characters might be entirely deceptive at times, for not only were the hair reminiscent of *Cynthia crinitistellata*, but one of the siphons was also much elongated, though in the present species it came out to be the atrial and not the branchial siphon

IV The Test

The test is thin, and soft and cartilaginous in section, its average thickness being only 1 mm It is almost smooth and transparent allowing the internal organs to be seen through At the place of attachment, however, the test is thrown into folds and appears to be corrugated There are a large number of branching vascular vessels in the test, while its matrix is interspersed with a number of minute, knob-headed, echinated spicules (Fig 3, microscleres) as described in *Herdmania pallida* by the author[3]

(1936). The test is traversed by the vessels which branch, anastomose, and send pear-shaped vascular ampullæ towards the surface. Each ampulla is lined by a single layer of cells which are largest at its distal and smallest at its proximal end No bladder cells could be discerned in the test. The inpushing of the test into each siphon is extremely thin and flecked with four white longitudinal streaks

V *The Internal Organs*

1 *The Mantle.*—The mantle or body wall is thick and muscular in the antero-dorsal half of the body, where it forms an opaque covering over the internal structures, but is very thin and transparent in the postero-ventral half, allowing the pericardium, the gonads, and part of the gut to be seen through it. At the antero-dorsal end the mantle is prolonged to

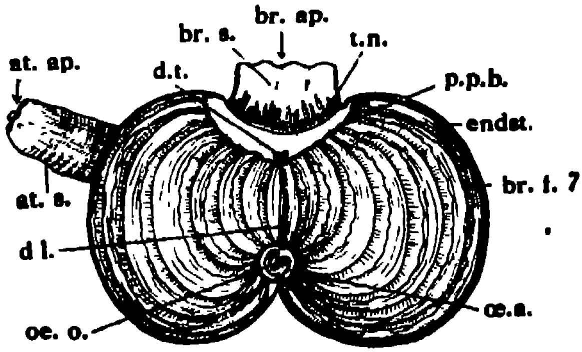

Fig. 1

The animal cut open longitudinally from the ventral side to show the internal view of the branchial sac (× 2). *at.ap*, atrial aperture; *at s.*, atrial siphon; *br.ap.*, branchial aperture; *br.f.* branchial fold; *br.s.* branchial siphon; *d.l.*. dorsal lamina; *d.t.*. dorsal tubercle; *endst.*, endostyle; *œ.a.*, oesophageal area; *œ.o.*, oesophageal opening; *p.p.b.*, peripharyngeal band: *t.n.*, tentacle

form two elongated siphons—the atrial much longer than the branchial—and the distal ends are produced into four lips which fit into four similar lips of the test The muscles of the mantle, as in *Herdmania pallida*, consist of (i) the branchial group, (ii) the atrial group, and (iii) the branchio-atrial group. At the place of their insertion the muscle bands of the longitudinal set in the branchial group number about 46, while in the atrial group they number roughly 34, the branchio-atrial group consisting of five muscle bands only, when compared to *H. pallida*, the numbers of which approximately are 60, 40 and 5 respectively

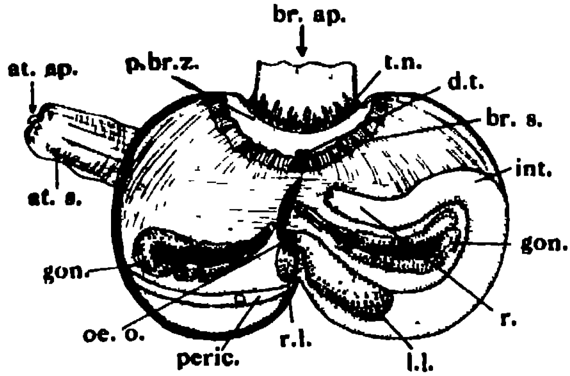

FIG. 2

A ventral view of the internal anatomy after removal of the branchial sac (× 2). *at.ap.*, atrial aperture; *at.s.*, atrial siphon; *br.ap*, branchial aperture; *br.s.*, branchial sac; *d.t.*, dorsal tubercle; *gon.*, gonad; *int* intestine; *l.l.*, left digestive gland; *oe.o*, oesophageal opening; *peric.* pericardium; *p.br z.*, pre-branchial zone; *r*, rectum; *r.l.*, right digestive gland; *t.n.*, tentacle.

A large number of spindle-shaped echinated calcareous spicules are present throughout the mantle, some of which even extend into the connective tissue of the internal organs As in *H pallida*, they are enclosed in tubular sheaths which run in long strings in the substance of the mantle (Fig. 3). The pipette-shaped spicules found in *H pallida* are absent in the present species

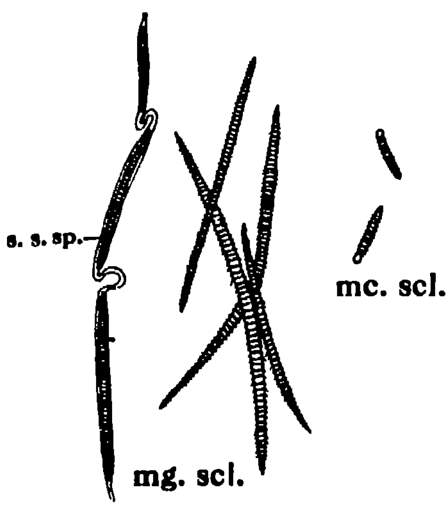

FIG. 3

The spicules of *Herdmania ennurensis* n. sp. (× 55). *mc.scl*, microscleres; *mg scl.*, megascleres; *s s.sp.*, spindle-shaped spicules.

2 *The Branchial Sac* —The branchial sac has 7 folds on each side, the ventral-most fold near the endostyle being the thinnest (Fig 1) There are 6 internal longitudinal bars (vessels) on a fold and 3 in the interspace between two adjacent folds, the bars being flattened inwards into thin flaps. The stigmatic areas in the interspaces are larger than those in the branchial folds and contain 8 to 10 stigmata each (Fig 5) Intra-stigmatic vessels are present There are three orders of transverse vessels in the wall of the branchial sac instead of four as in *Herdmania pallida*

3 *The Tentacles* —There are 22 to 24 tentacles, in three sizes (Fig. 4), about 8 being large, 8 medium, and 8 small-sized, those of various sizes

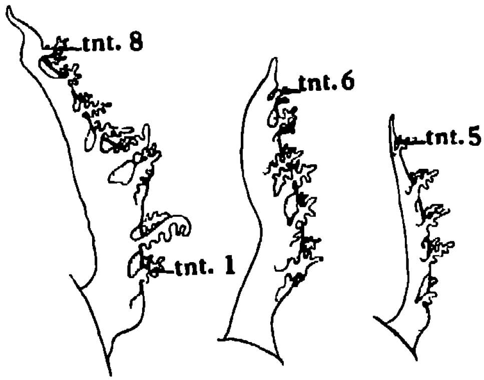

FIG. 4

The three orders of tentacles viewed from the side (× 12). *tnt.*, 1-8, first to eighth tentaculet.

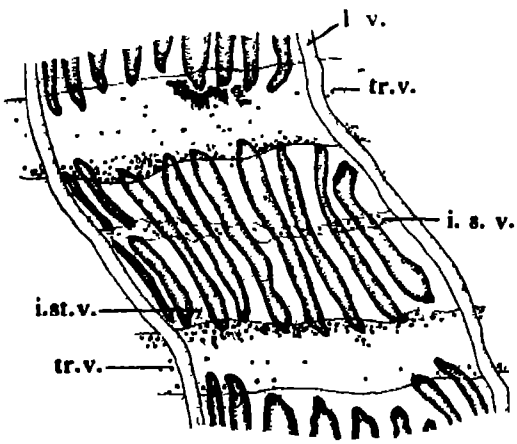

FIG. 5

A part of the branchial sac to show the branchial vessels and the stigmata (× 56). *i.s v.*, intra-stigmatic vessel; *i st v.*, inter-stigmatic vessel; *l.v*, longitudinal vessel, *tr.v.*, transverse vessel.

alternating with one another The tentacles are much branched and of the type called 'compound' by Herdman Each tentacle is a flat curved structure attached by a broad base to the inner wall of the branchial siphon in the region of the branchial sphincter The anterior border of the tentacle carries two rows of lateral branches, while the posterior portion forms a thin membraneous flap (Fig 4) The branches or tentaculets carry two rows of secondary branches, but the tertiary branches of *H pallida* are absent here

4 *The Dorsal Tubercle* —The dorsal tubercle is comparatively large for a small ascidian of the present genus It consists of a broad base from which arise two conical projections, each of which is formed by a spirally coiled conical lobe (Fig 6) Each spirally coiled lobe consists of two loops or coils (3 in *H pallida*) the proximal one being larger.

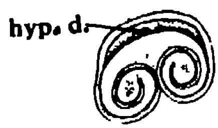

FIG. 6

Vertical view of the dorsal tubercle (× 26). *hyp d*, opening of the duct, of the Hypophysial (neural) gland.

5 *The Peripharyngeal Bands* —The peripharyngeal bands have a characteristic undulating course, being convex at the places where the branchial folds arise, and pushed inwards at the intervening spaces (Fig 1).

6 *The Dorsal Lamina* —The dorsal lamina is about 5 mm in length and consists of 18 to 20 dorsal languets borne on a narrow flap attached to the mid-dorsal wall of the branchial sac (Fig 1). Each languet is broadest at its base and tapers to a point at its distal end (Fig. 7) The languets are longest in the posterior third of the dorsal lamina

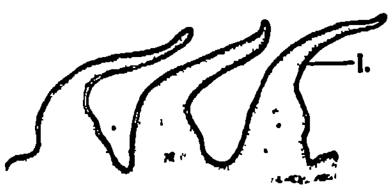

FIG. 7

Side view of a part of the dorsal lamina with languets (× 55) *l.*, languet.

7 *The Alimentary Canal* —The œsophageal aperture (Fig 1), surrounded by the œsophageal area, leads through a short œsophagus into a wide tubular stomach. There are two digestive glands (Fig 2), the larger situated on

the left and the smaller on the right side of the stomach, into which they open The left gland measures 8 mm. × 3 mm and the right 3 mm. × 3 mm. approximately The intestine forms a long wide loop on the left side of the body, and the rectal orifice has four lips.

8 *The Gonads* -- There are two lobed gonads (Fig 2), one on each side of the body Each gonad along with its duct, measures roughly 12 mm. by 4 mm., the width representing the broadest posterior region of the gonad. The right gonad is usually more or less smaller than the left one The testicular cæca are arranged in 9–14 clumps all around the outer side of each gonad, while the ovarian follicles lie along the central axis

VI. Specific Characters

The size of the present species is smaller than that of other species of *Herdmania* The test is thin, almost transparent, and devoid of sand, shell-fragments or spines and hair. The atrial siphon is very long, almost equal to half the length of the body, and the siphonal apertures are situated wide apart The compound tentacles are of three alternating sizes and are usually 24 in number The dorsal tubercle is large, consisting of two spiral coils. The stigmatic areas contain 8 to 10 stigmata each, intra-stigmatic vessels are present

VII Remarks

Herdman[6] (1891) described 10 species of *Herdmania* (*Rhabdocynthia*), *viz*, *H mollis*, *H sacciformis*, *H mauritiana*, *H subfusca*, *H tenuis*, *H papietensis*, *H complanata*, *H rosea*, *H pyriformis* and *H pallida*, of which only two (*H mauritiana* and *H pallida*) were reported from the Indian Ocean Later[5] (1906) he added one more species (*H ceylonica*) to this list. The species described in this paper brings the number of species of *Herdmania*, found in Indian waters, to four

According to the detailed examination made by the author[2] (1936), the number of folds in the wall of the branchial sac is more or less a constant character in all the species of Pyuridæ Only 3 Pyurids have yet been described with 7 branchial folds, *viz*, *H subfusca*, *H tenuis* and *H ceylonica* *H. ennurensis* also has 7 folds in its branchial wall on each side of the body It differs, however, from *H. subfusca* in its pear-shaped body, in its smaller size, in the number of tentacles, and in the number of stigmata in each stigmatic area The present species differs from *H tenuis* in the size and shape of its body, in the number of tentacles, and in the arrangement of stigmata. It also differs from *H ceylonica* in the absence of sand or shell fragments from the test (though much importance should not be attached

to this character, as the presence or absence of sand or shell-fragments depends mostly upon the nature of the substratum), in the great length of the atrial siphon, and in the large size of the dorsal tubercle with 2 spiral coils

Herdmania is a cosmopolitan Pyurid found in all the seas of the world, except the Arctic of which the ascidian fauna has not been properly investigated The Indian species *H pallida* has been reported from the Pacific and Atlantic Oceans, as also from Malaya and West Indies The other two Indian species *H mauritiana* and *H ceylonica* have been reported only from the Indian Ocean ; while the present species *H. ennurensis* should be common on the East Coast of India

VIII Summary

The author describes a new species of *Herdmania* (fam Pyuridæ) from Ennur (Madras) The present species can be distinguished from other existing species of *Herdmania* by the co-existence of the following characters ·

Body small and pear-shaped , atrial siphon enormously elongated, being almost equal to the length of the body ; dorsal tubercle large, consisting of a double spiral , tentacles compound, in 3 alternating sizes and 24 in number , branchial sac with 7 folds on each side , 8–10 stigmata in each stigmatic area.

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KEY TO THE INDIAN SPECIES OF *HERDMANIA*

Branchial sac with 7 folds on each side—1.

Branchial sac with more than 7 folds on each side—2.

1. Dorsal tubercle simple horse-shoe shaped —*H. ceylonica*.
   Dorsal tubercle consisting of a double spiral—*H. ennurensis*.
2. Branchial sac with 8 folds on each side—*H. mauritiana*.
   Branchial sac with 9 or 10 folds on each side—*H. pallida*.

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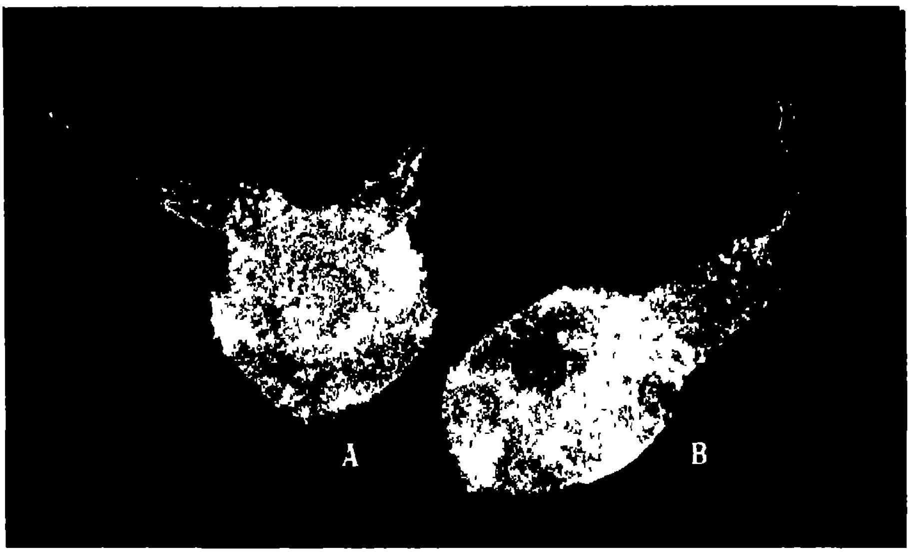

FIG. 8

Photograph of two entire specimens of *Herdmania ennurensis* n. sp. *A.*, side view ; *B.*, dorsal view ; (× 1½ nat. size).

SOME STUDIES ON THE METABOLISM AND GROWTH OF MALTA ORANGES

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Introduction

It is now recognised that the study of metabolic activities of fruits during their whole course of development furnishes such important data as can be very helpful for explaining the changes in the body of ripened fruits exposed to conditions of cold and gas storage Recently Kidd and West (1924), Wardlaw and Leonard (1935) and B N Singh (1937) have published a comprehensive account of the respiratory activity of apples, papaw and mangoes respectively during the period of growth of the fruits

In this note an account is given of the metabolic drift of malta oranges. Citrus is the main fruit of the Punjab plains and malta orange occupies a large area in the canal colonies

Orange juice is considered very good for health and is most suitable for invalids The fruit is in great demand throughout the year The storage of oranges has therefore great economic value, for making them available in summer Physiological studies are important for determination of the length of time for which the fruit can be stored without any adverse biochemical changes

Material and Method

The material required for the investigation during different phases of development of the fruit was obtained from trees of the " Common malta type " growing in the experimental fruit garden of the Agricultural College and Research Institute, Lyallpur A large number of fruits was marked soon after setting. They were picked from the same tree at fortnightly intervals and carried to the Laboratory for investigation

Respiration.—The output of carbon dioxide was measured by the continuous current method using 0·5 N, NaOH solution as absorbent in specially designed bubblers (Luthra and Chima, 1931) Parallel sets were always run at 30° C for 24 hours. The respiratory activity was expressed in terms of mg of carbon dioxide given out per hour per 100 gm of fresh weight of fruit.

Growth Rate —The increase in dry weight of the fruit at successive intervals has been mathematically interpreted and the growth rate calculated by the formula (Blackman, 1919, 1920) $R/100 = Log_e\, w_1 - Log_e\, w_0$, where w_0 and w_1 are the dry weights of two successive fortnights and e the base of natural logarithm

Nitrogen —Nitrogen was estimated by the Skinner (1930) modification of Kjeldahl's method

Sugars —For the estimation of sugars five fruits were selected and cut up into small pieces and thoroughly mixed Duplicate samples consisting of 50–60 gm were taken for alcoholic extraction in a Soxhlets extraction apparatus A little of ammonia was always kept in receiving flasks to neutralize the acids After removing alcohol from the alcohol-soluble material under reduced pressure, the residue was taken up in water clarified, deleaded with sodium phosphate and made up to volume. Reducing sugars were estimated volumetrically using Lane and Eynon's method (1923)

The total sugars were estimated as reducing sugars after inversion of 50 c c of the above solution by 10% hydrochloric acid and neutralizing the acid with sodium carbonate. The values for sucrose were obtained by the difference between total sugars and reducing sugars

Total Titrable Acids —The total titrable acid was estimated as citric acid in the water extract obtained by ramming a known weight of the fresh fruit in a pestle-mortar and titrating the aliquot portions against N/10 NaOH

Total Solids —A requisite amount of material was dried in an oven at 90° C to a constant weight. In later stages of development of the fruit drying was accomplished under reduced pressure at low temperature to obviate loss of sugars, etc.

Results

Respiration —The measurement of the respiratory activity of the malta orange fruit was begun at an early stage when the fruit was only 5 days old and was followed up till the fruit matured. The results obtained are given in Table I and Fig I

TABLE I

Showing the respiratory activity of the malta orange fruit during the major phases of its development throughout the life-cycle at 30° C and percentage Nitrogen on dry weight basis

Age of fruit in days (after setting)	Mg. of CO_2 evolved per 100 gm. of fresh fruit per hour	Percentage nitrogen on dry weight basis	Remarks
5	75 56	2 596	Fruit very young, colour dark green.
20	49·92	2 468	
35	35 36	2 345	
50	32·26	2 170	
65	20·30	2·100	
80	12·47	2 150	Fruit developing in size; colour dark green.
95	10 75	1·890	
110	8 32	1 715	
125	7 96	1 610	
140	7 29	1 592	
155	7·11	1 965	
170	5 77	1·820	
200	6·05	.	Colour changes from green to greenish yellow
230	10·00	1 778	
245	15 00	1·776	
260	4·50	..	Fruit matured and colour was orange yellow.
290	3·75	..	

The results obtained corroborate the findings of Kidd (1934), Wardlaw and Leonard (1935), B. N. Singh (1937), and Luthra and China (1931). The fruit when young is metabolically very active and gives out CO_2 at the rate of 75·56 mg. per 100 gm of fruit per hour. Subsequently as the fruit ages

and advances in size, a definite rapid fall characteristic of respiration of fruits is noticeable and is followed by a more steep fall till the 170th day when the respiration rate is reduced to 5·77 mg per hour The respiratory

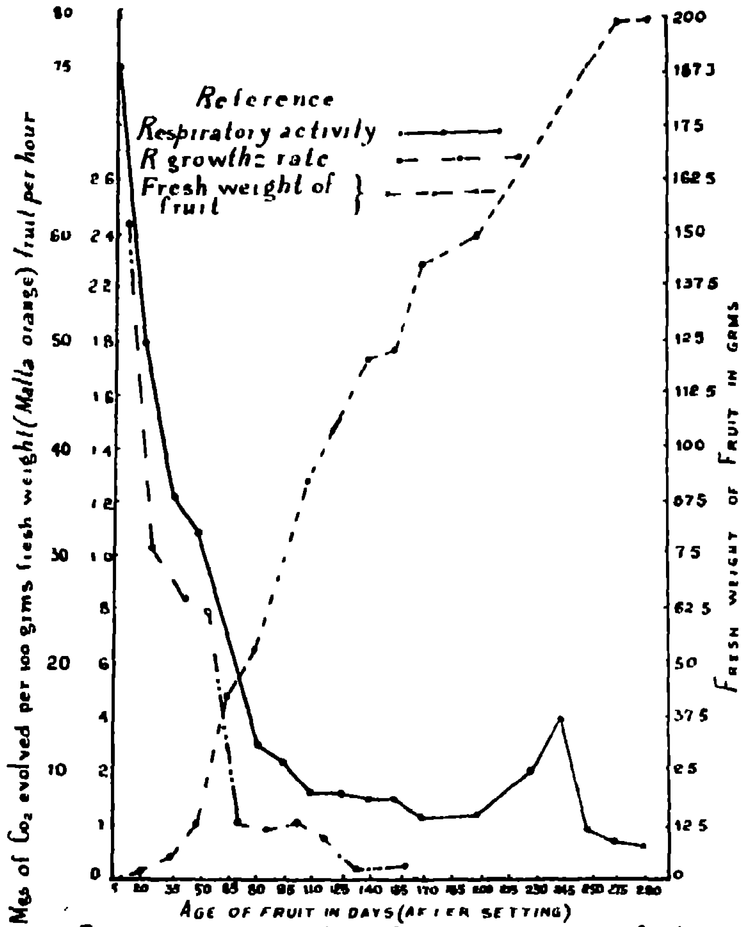


Fig. 1 Respiratory activity throughout life per 100 grms. fresh weight (Malta orange) Fruit and Relative growth rate

activity again rises to a second maximum (15·00 mg. per hour) at 245th day after setting This climacteric rise (Kidd, 1934) is concomitant with the onset of change of colour of the fruit from green to greenish yellow The respiration again slows down towards the end of the life of the fruit It has been determined upto 290th day and at that stage it is only 3·75 mg per hour per 100 gm.

Relative Growth Rate —Relative growth rate of the fruit (Fig. I and Table II) is highest (2·42) during the early period of the life-cycle and decreases rapidly till it becomes almost constant towards the end.

TABLE II

Showing relative growth rate of the malta orange fruit during development

Age of fruit in days	Absolute dry weight of fruit in gm	$\mathrm{Log}_e\, w$	Relative growth rate $\mathrm{Log}_e\, w_1 - \mathrm{Log}_e\, w_0$
5	0·275	−3·59357	2 42882
20	0·312	−1 16475	1·21936
35	1·058	0·05461	1·02685
50	2 949	1 08146	0·97763
65	7 839	2 05909	0 19378
80	9·515	2·25287	0·17802
95	11·370	2 43089	0·20113
110	13 902	2 63202	0 13238
125	16 030	2·77440	0 02530
140	16·440	2·79970	0·02197
155	16·805	2 82167	0·04005
170	17 490	2·86162	

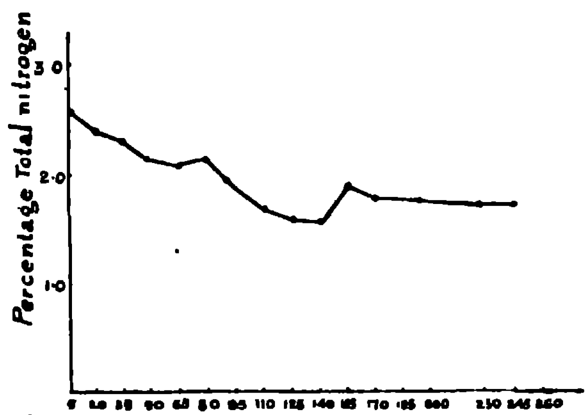


FIG. II

PERCENTAGE TOTAL NITROGEN IN MALTA-ORANGE FRUIT EXPRESSED IN TERMS OF DRY WEIGHT

Chemical Changes in the Developing Fruits

Carbohydrates —During growth of the malta orange fruit reducing sugars (Table III and Fig III) show a rapid increase in the first month Afterwards the increase is gradual and the maximum is attained on the 170th day after setting, when the amount actually present is 12·85% At this stage the respiratory activity is at its lowest This period when fruits contain the

TABLE III

Showing percentage of carbohydrates, Total Acids, and Total Solids during the life-cycle of the malta orange fruit

Days after setting	Carbohydrates			Total Titrable Acids	Total solids	Per cent. Moisture
	Reducing sugars	Sucrose	Total sugars			
5	Nil	Nil	Nil	Nil	33·33	66·67
20	3·02	Absent	3 02	3 00	26·73	73·27
35	6·796	1·375	8 171	3 30	21 04	78 96
50	6 810	1·730	8·540	3·818	21 65	78·35
65	7·832	4·082	11 914	4 70	18 47	81·53
80	8·60	4 60	13 20	..	18·14	81·85
95	10·67	5·13	15·80	5·86	15·67	84·41
110	11·14	7 49	18 63	6 00	14·70	85·30
125	10 69	9·34	20 85	6·93	15·05	84 94
140	10 0	10·6	20·6	6·98	13 70	86·29
155	11·2	15·2	26·4	7·00	12·54	87·46
170	12·85	15·72	28·57	7 50	12·28	87·72
200	12·25	16·82	29·07	7·20	8 08	91·92*
230	12·20	17·00	29·20	3·40	8·74	91·26†
260	12·03	17·10	29·13	3·10	..	..

* Colour changes from green to greenish yellow.

† Fruit fully matured.

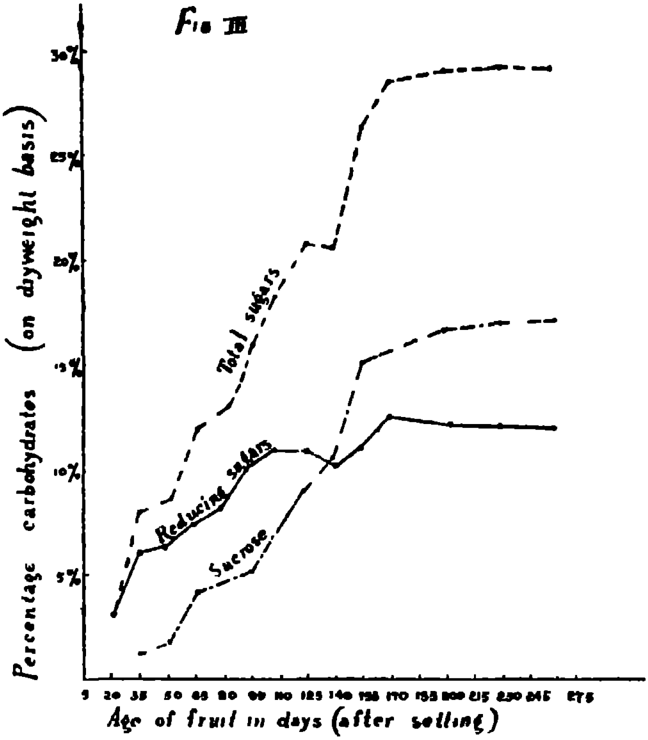


Fig III
30%
25%
20%
15%
10%
5%
Total sugars
Reducing sugars
Sucrose
Percentage carbohydrates (on dryweight basis)
Age of fruit in days (after setting)

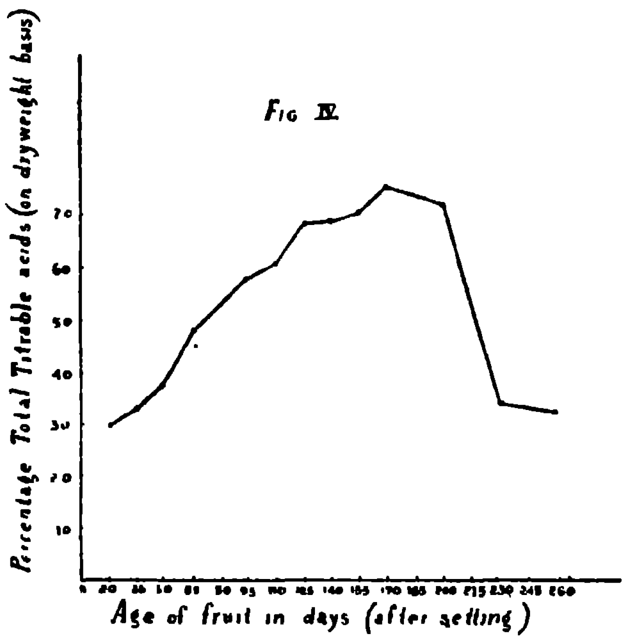
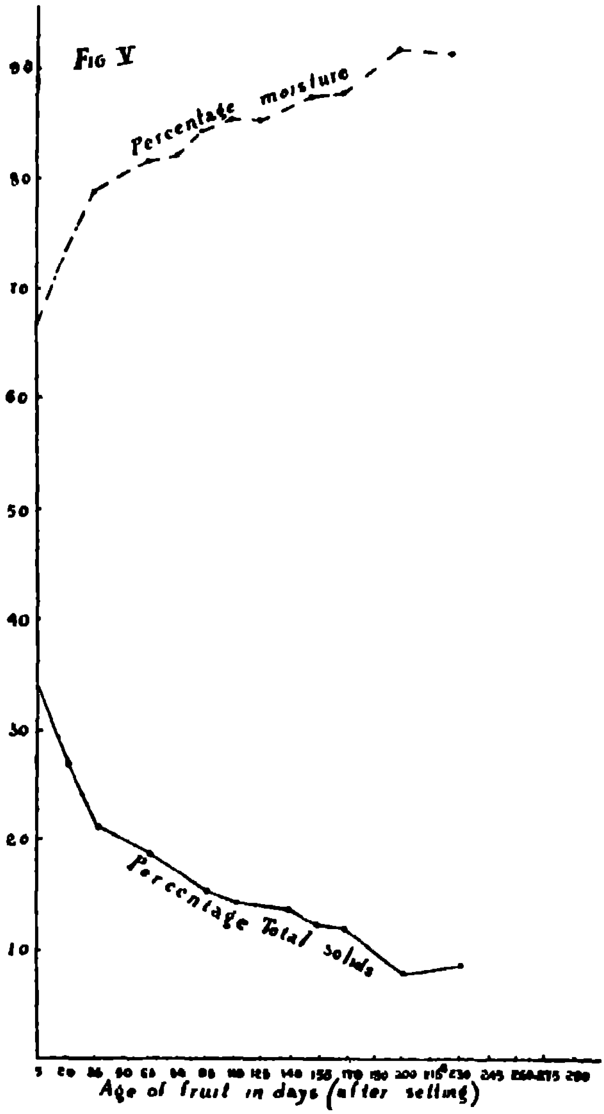


Fig IV
70
60
50
40
30
20
10
Percentage Total Titrable acids (on dryweight basis)
Age of fruit in days (after setting)

highest amount of hexoses coincides with the period when the fruit is just on its way to climacteric rise of respiration and change of colour from green to greenish yellow. A gradual decrease in the reducing sugar contents towards maturity is noticeable and falls in line with the decreasing respiratory activity during the senescent stage

Sucrose is found to accumulate consistently (Table III and Fig III) throughout the life-cycle of the fruit and the maximum is reached at maturity



Total Titrable Acids.—The acid contents of the fruit steadily increase till 170th day (7·5%) after which they begin to decrease as the fruit matures (Table III and Fig IV)

Total Nitrogen.—The fruits when young are richer in nitrogen contents (Fig. II), which fall down subsequently as the fruit ages

Total Solids.—Total solids (Table III and Fig. V) are highest during early stages of the development of the fruit but decrease gradually with progressive hydration

Discussion of the Results.—The highest respiratory activity and the highest growth rate (Fig I) during the adolescent stage may be associated with the increased activity and rapid formation of new tissues (B N Singh, 1937) and (Kid, 1934) It is also interesting to note the parallelism between the respiratory activity, gorwth rate and nitrogen (Table I and Fig. II). According to Palladin (1922) high nitrogen values are associated with greater protein-nuclein content of the protoplasm, and probably a higher rate of growth and higher metabolic activity of the fruit are also connected with it (Archbold, 1925)

The coefficient of correlation between (1) respiratory activity and nitrogen (+ ·913 ± ·044), (2) respiratory activity and growth rate (+ ·985 ± ·099) and (3) nitrogen and growth rate (+ ·880 ± ·070) is very high and positive

The subsequent slowing down of the respiraton rate, despite the formation and accumulation of sugars (Table III and Fig. III) seems to be due to the development of more fibrous tissue as the fruit gorws The increase in the thickness of the epicarp from 1 06 mm when the fruit is young to 6 85 mm at maturity may also be responsible for the poor ingress of atmospheric gases and subsequent poor output of CO_2 in the later stage The absence of stomata and lenticels on the surface of the skin of the fruit points to the entrance of O_2 and its subsequent distribution into the interior by surface diffusion The second high value of respiratory activity, which occurs at the climacteric stage marks the onset of colour changes in the fruit from green to greenish yellow and finally to orange yellow and is similar to that observed by Kidd and West (1924) and Wardlaw and Leonard (1935) and B N Singh (1937)

Summary

1. The respiratory activity, relative growth rate, nitrogen content and the march of carbohydrates and acidity have been studied throughout its development from adolescence to maturity

2 A curve characteristic of the respiration of fruits has been obtained and the results are in accord with the findings of Kidd, Wardlaw and Leonard and B N Singh The respiration intensity is very high during the adolescent stage and falls off rapidly, prior to second maximum which marks the onset of colour changes in the fruit during maturation.

3 The growth rate and nitrogen content are highest during the earlier stages and decline continuously towards the end

4 The respiratory activity, growth rate and the nitrogen values run parallel to each other and statistical studies exhibit a very high and positive correlation between them

5 Reducing sugars, sucrose and total sugars steadily accumulate from adolescence to maturity The reducing sugars however show a decline towards the end and are highest just before the onset of climacteric rise in respiration.

6. The total titrable acids gradually increase till 170th day, but fall down later on

7 The total solids are highest in the beginning when the fruit is young but later on decrease on account of the progressive hydration which follows subsequently.

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THE VENOUS SYSTEM OF THE POND-TURTLE, *LISSEMYS PUNCTATA (BONNATERRE)**

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Received December 7, 1939

(Communicated by Mr. Beni Charan Mahendra, F.Z.S.)

Introduction

There is a great paucity of detailed information about the venous system of turtles and many families have yet to be worked out carefully before we can get a sound comparative knowledge of this system in this group. Amongst the older workers, we need mention only Bojanus (1819–21), Rathke (1848) and Nicolai (1826), of which the last has given a detailed description of the renal portal system in *Testudo orbicularis* (*Emys europæa*) Amongst the later authors, Burne's account of the leathery turtle *Dermochelys coriacea* (1905), Bruner's work on the cephalic vessels of *Emys europæa* (1907), Stromsen's study on the anatomy and development of the venous system of turtles (1905), and Grodziński's work on the development of the blood vessels in the fore-limbs of *Emys orbicularis* (1930) stand out conspicuous. As far as I have been able to ascertain, no Indian turtle has so far been studied from this standpoint

The pond turtle *Lessemys punctata* (*Bonnaterre*) is one of the commonest Indian Chelonians and is practically found all over the country. It is of quiet disposition and can be readily obtained Its plastron is united to the carapace by ligamentous tissue and does not offer such a great difficulty in removal as the one experienced in the turtles belonging to the family *Emydidæ* (*Kachuga, Hardella, Morenia*, etc). Its internal anatomy is fairly typical, and it fully illustrates the kind of organisation met with in the order *Testudines* All these features make it an excellent type for class dissections, and it is really desirable to have a detailed knowledge of its anatomy and development.

Material and Technique

More than a dozen specimens of the pond-turtle were used for the present study The species (*Lissemys punctata, forma typica*) was determined for me by Mr Beni Charan Mahendra The individuals including both sexes varied in length of carapace from 5 inches, or slightly less, to 9½ inches

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* Work done under the direction of Mr. Beni Charan Mahendra at the Department of Zoology, St. John's College, Agra.

Besides dissections of fresh specimens, which show almost all the major veins distinctly, the following injection-mass was used for tracing the various veins more completely

Water	..	..	..	100 c c.
Glycerine	..	..	..	20 c.c
Formaline (40%)		..	..	20 c.c.
Powdered corn starch		..	..	75 gms.
Methylene Blue		..	..	10 gms.

The Precaval Veins

There are two *precaval veins* (*Venæ anonymæ*), one right and the other left, opening into the anterior part of the *sinus venosus*.

The *right precaval* (Text-Fig 1) is formed by the union of two veins; the *Vena subclavia* and the *Vena jugularis*.

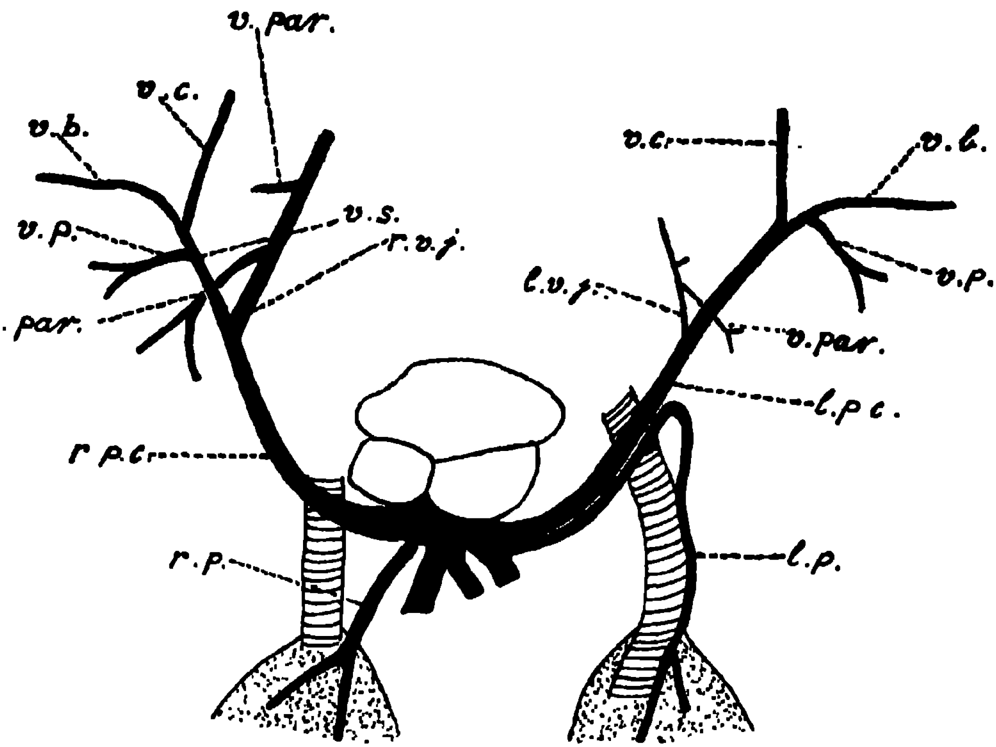

Fig. 1

The Anterior Veins of *Lissemys punctata* (Ventral aspect)

l.p., left pulmonary vein ; *l.p.c.*, left precaval vein ; *l.v.j.*, left jugular vein ; *r.p.*, right pulmonary vein ; *r.p.c.*, right precaval vein ; *r.v.j.*, right jugular vein ; *v.b.*, vena brachialis ; *v.c.*, vena cutanea ; *v.p.*, vena pectoralis ; *v.par.*, vena parietalis ; *v.s.*, vena subclavia.

The *Vena subclavia* is composed of three main branches:—

(*a*) *Vena cutanea*, which comes from the skin covering the depression between the carapace and the plastron for the accommodation of the right fore-limb

(*b*) *Vena brachialis*, which collects the venous blood from the right fore-limb.

(*c*) *Vena pectoralis*, which is formed by the union of two veins running over the right half of the pectoral girdle

The *Vena jugularis* is a large vein bringing blood mainly from the head, neck and the associated parts, but it also receives, a little before joining the *Vena subclavia*, two veins from the shell The anterior one receives blood from the antero-ventral aspect of the carapace, while the posterior one is formed by the union of a vein from the marginal parts of the carapace and another from the vertebral region The latter vein (*Vena azygos*) runs longitudinally on the right side of the vertebral column and receives blood from a number of intervertebral veins

The *left precaval* is formed, on the whole, on the same plan as the right but the jugular vein of this side is much narrower than the right one On account of this disproportionate development of the two jugulars, the venous blood from the head and the neck goes to the heart mainly by means of the right precaval, the left one only conducting a small amount I have not been able to find any previous record of such an asymmetry in the jugular veins of the two sides

The Veins of the Head and Neck

Traced towards the head (Text-Fig 2), the two jugular veins are connected to each other, near the base of the neck, by a transverse anastomosis. The right jugular vein soon occupies a mid-dorsal position, receives tributaries from the muscles of the cervical region, and has a longitudinal vein opening into it on the right side near the anastomosis From this point forwards to the posterior part of the head we have three longitudinal veins running side by side on the dorsal side of the neck, the middle one being the right jugular.

All these lead into a large transverse sinus situated at the hinder aspect of the head and receiving tributaries from its various parts. The more important veins of the head on each side are --

(*a*) The *Vena mandibularis*, running along the two rami of the mandibles and receiving blood from the lower jaw

(*b*) The *Vena hyoidea*, opening into the mandibular vein and bringing blood from the hyoid and its neighbourhood

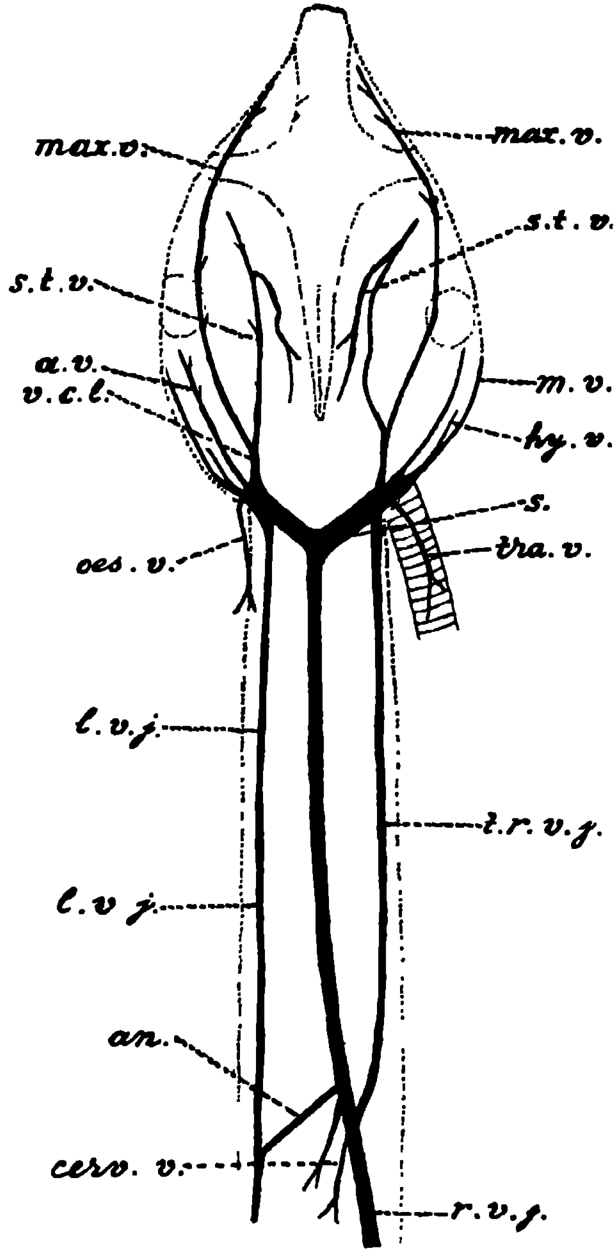

Fig. 2

The Veins of the Head and the Neck (Dorsal view)

an., anastomosis ; *a.v.*, auricular vein ; *cer.v.*, cervical veins ; *hy.v.*, vena hyoidea ; *m.v.*, vena mandibularis , *max.v*, vena maxillaris , *œs.v.*, vena œsophagea ; *s.*, sinus at back of head ; *s.c.v.*, vena supratemporalis , *t.r.v.j.*, right longitudinal tributary of right jugular vein , *tr.v.* tracheal vein ; *v.c.l.*, vena capitis lateralis (other letters as in Fig. 1.)

(*c*) The *Vena capitis lateralis*, formed by two important veins, one (*Vena maxillaris*) collecting blood from the maxillary region, and the other (*Vena supratemporalis*) distributed over the muscles of the temporal region

(*d*) The *Venæ palatines*, distributed over the palate

(*e*) The *Vena œsophagea*, a delicate vein from the anterior part of the œsophagus

(*f*) The *Vena trachealis* from the anterior part of the trachea

The Posterior Venous Supply

The venous blood from each hind foot (Text-Fig 3) is collected by several small veins, which unite together to form a single vein running up the limb

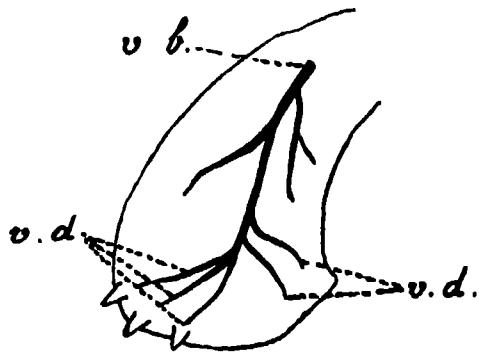

Fig. 3

The Veins of the Right Limb

v.f, vena femoralis, *v d*, venæ digitales

and receiving one or two more veins from the shank The common vein thus formed, the *vena femoralis*, runs along the posterior part of the thigh and divides into two branches near the acetabular region (Text-Fig 4).

One of the branches of the *vena femoralis* runs forwards in an obliquely upward direction to open into the lateral lobe of the liver and is apparently the vessel called the ' anterior abdominal vein ' by some authors As, however, the anterior abdominal vein in other vertebrates is a single vessel formed by the union of the two pelvic veins, it seems more reasonable to regard these vessels in the Chelonia as corresponding to the un-united pelvics, rather than to the anterior abdominal vein In order to obviate the suggestion of homology unconsciously assumed in nomenclature, I prefer to call these veins as *lateral abdominal* or *epigastric veins*

As is generally known, the presence of two separate abdominal veins is typical of Chelonia. Rathke (1848), however, observes the absence of

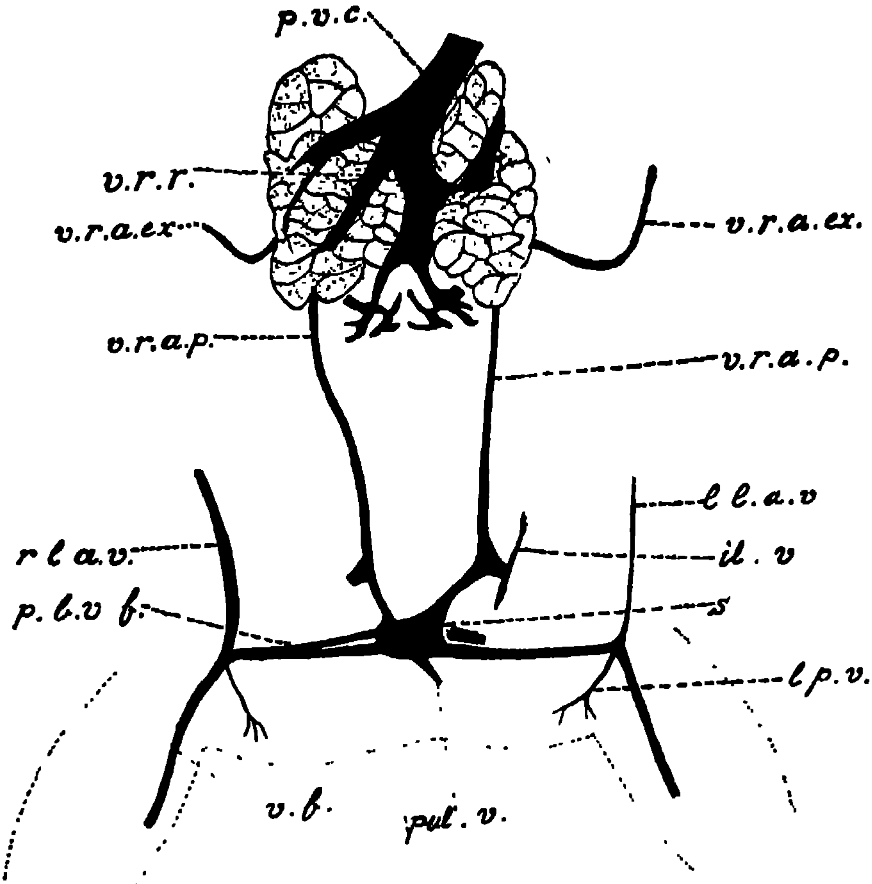

FIG. 4

The Posterior Veins (Ventral view)

il.v., vein from the iliac region, *l l.a v*, left lateral abdominal vein, *l p v*, ventrolateral pelvic vein, *p b v f*, the pelvic branch of the vena femoralis; *pub v*, pubic vein, *p v.c.*, posterior vena cava, *r.l a.v*, right laleral abdominal vein, *s.*, pubic sinus, *v f.*, vena femoralis, *v r.a e x*, vena renalis advehens externa, *v.r.a p.*, vena renalis advehens posterior, *v.r.r.*, vena renalis revehens.

the right abdominal vein in young individuals of *Chelone* and in his specimen of *Dermochelys* Burne (1905) finds that the right abdominal vein in *Dermochelys* is present, being represented by a small vessel formed by the union of the veins from the muscles of the right coracoid and entering the posterior edge of the isthmus of the liver about its middle He compares this condition with that of *Testudo græca*, in which the right abdominal vein may be quite insignificant. In *Lissemys punctata*, I find the reverse condition. The right abdominal vein here is fully developed, while the left is extremely small.

The second branch of the *vena femoralis* runs towards its fellow of the other side to form a large sinus, receiving several minute vessels from this region and a large vein from the pubic portion of the pelvis From the posterior part of this sinus, two veins (the *venæ renales advehentes posterior*) take their origin, run upwards and forwards, receiving a vein from the iliac region and finally enter the kidneys at their posterior end

As described by Nicolai, the *vena renalis advehens posterior* in *Testudo orbicularis*, quite unlike the condition found by me in *Lissemys punctata*, has no connection with the vein from the posterior part of the thigh or with the abdominal vein. *The occurrence of a pubic sinus in turtles has not been previously recorded*

The *Vena femoralis*, just at its point of bifurcation to form the foregoing two branches, receives a vein from the antero-lateral region of the pelvis

Besides the *venæ renales advehentes posterior* described above, each kidney receives two more veins--the *vena renalis advehens externa* and the *vena renalis advehens anterior* The *vena renalis advehens externa* brings blood from the posterior part of the shell and the muscles associated with it It runs on the inner aspect of the lateral border of the carapace and receives transverse veins bringing blood from the inner aspects of both the carapace and the plastron

The *vena renalis advehens anterior* is formed by the anterior and middle veins of the shell and runs longitudinally lateral to the vertebral column It opens into the antero-dorsal aspect of the kidney and is not visible in a ventral view

The blood from the kidneys is collected by three or four large veins, the *venæ renales revehentes*, which unite to form the *posterior vena cava* going forwards to the heart and traversing the liver on its way There are two peculiarities about the *posterior vena cava* worth noting In the first place, it lies asymmetrically, distinctly inclined towards the left side as it goes forwards, being adpressed to the anterior border of the left kidney This is apparently a primitive type of arrangement As is well known, the *posterior vena cava* of vertebrates arises at first on the left side in connection with the left kidney and becomes symmetrically placed in the median line only later on The disposition of the *posterior vena cava* to the left in *Lissemys punctata*, therefore, is an important primitive feature

Secondly, the *posterior vena cava* is continued backwards even behind the hinder borders of the kidneys and collects blood from the dorsal parietes in this region

The Liver and its Veins

The liver of *Lissemys punctata* is a large organ, much broader than long and divided into a smaller left lobe and a larger right one (Text-Fig 5) The

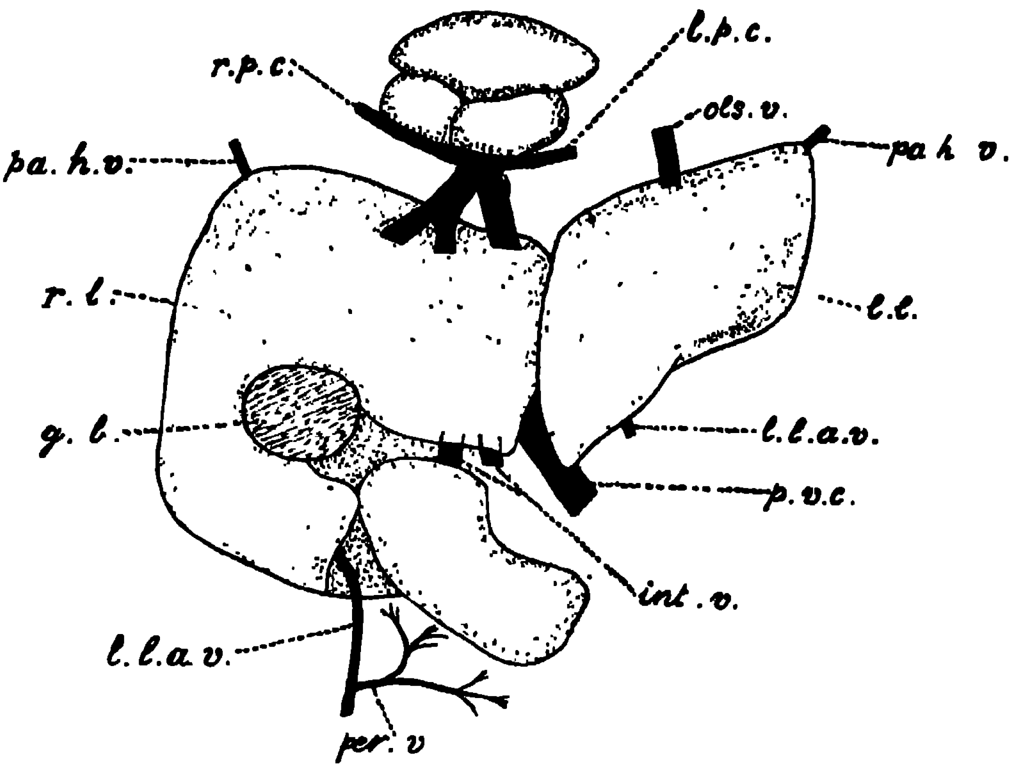

FIG. 5

The Veins in connection with the Liver (Ventral view)

g.b., gall bladder, *int v*, intestinal veins, *l.l.*, left lobe of the liver; *œs v.*, œsophageal vein, *pa h.v.*, hepatic tributary from the shell, *per v*, peritoneal tributary to the lateral abdominal vein, *r.l*, right lobe of the liver. (The other letters as in previous figures.)

gall-bladder lies embedded on the ventral side of the right lobe, and the ventral surface of the liver is depressed to accommodate the spleen and the intestine

The *posterior vena cava* enters the right lobe of the liver on the ventral aspect of its hinder border near the line separating it from the left lobe, and comes out at the ventral aspect of the anterior border of the right lobe

The *hepatic veins* are paired Instead of joining the *posterior vena cava* near the liver, they unite together to form a common stem, opening into the *sinus venosus* near the root of the posterior vena cava This primitive arrangement, as far as I can ascertain, has not previously been noted Each lobe of the liver receives a vein, at its antero-lateral end, from the shell and its muscles.

A large vein, the *œsophageal vein*, collects blood from the œsophagus and opens into the anterior border of the left lobe

Two large veins, the *intestinal veins*, open into the ventral aspect of the right lobe at the middle of the anterior border. These veins run alongside of the intestine

The *left abdominal vein* opens into the hinder border of the left lobe of the liver; the right, into the right lobe in the region situated behind the gall-bladder.

The continuance of the *hepatic veins* separately from the *posterior vena cava* right up to the *sinus venosus*, and the presence of many separate veins from the alimentary canal to the liver are features worth noting They appear to be reminiscent of the primitive condition in vertebrates, when the hepatic veins (originally, the *omphalo-mesenteric veins*) opened directly into the sinus venosus, instead of joining the *posterior vena cava*, which is a vessel phylogenetically more recent than them

The Veins of the Shell

Lying along the ventral aspect of the outer borders on each side of the carapace (Text-Fig 6), there is a longitudinal vein of considerable size, the *marginal vein of the shell*, receiving tributaries both from the carapace and the plastron. Anteriorly it is in communication with the *vena subclavia*, and posteriorly is united by means of two connecting veins with the *vena renalis advehens externa*. It receives a transverse vein (the *intercostal vein*) from each intercostal region of the carapace and numerous small veins, on each side, from the borders of the carapace and from the ventral aspect of the plastron.

The *marginal vein* receives anteriorly a vein, the *vena vertebralis*, which runs longitudinally dorsal to the roots of the ribs on either side of the vertebræ, and can be traced right upto the last vertebra of the carapace Close and parallel to the *vena vertebralis* there is another vein on each side, which arises anteriorly as a slender vessel, is epaxonic in position like the vertebral vein, and turns downwards and inwards, between the fifth and sixth rib of the shell It enters the anterior border of the kidney and extends over its dorsal region This is the *vena renalis advehens anterior*

As already mentioned, the liver also receives a vein from the shell on each side towards its antero-lateral end. This vein is a tributary coming from the lateral vein of the shell (Text-Fig. 5)

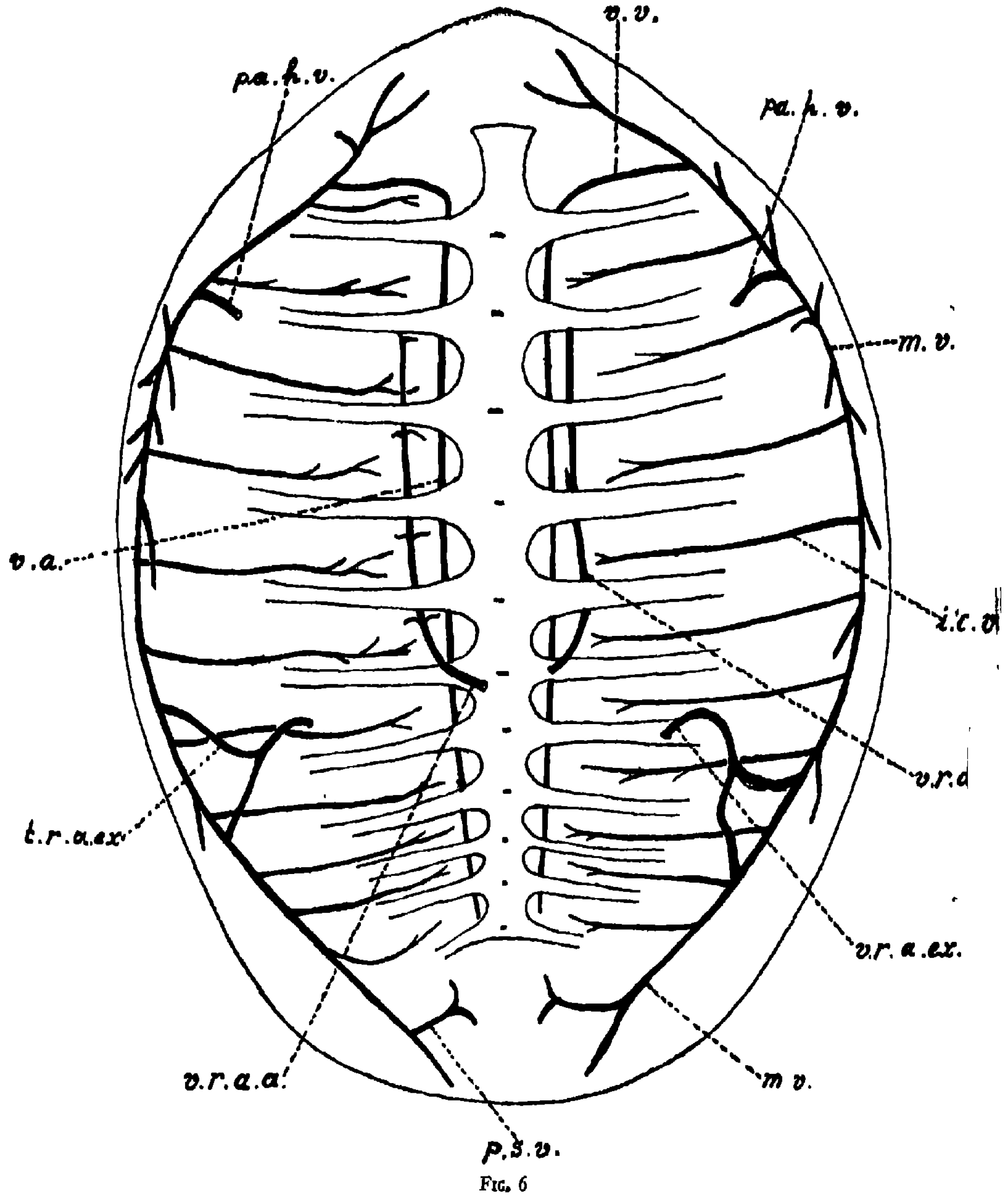

FIG. 6

The Veins of the Carapace (Ventral aspect)

i.c v, intercostal veins, m.v, marginal vein of the shell, p.s v., posterior vein of the carapace; p a.h v, parieto-hepatic vein, t.r.a ex, tributary from the marginal vein of the shell to the vena renalis advehens externa, v a., vena azygos, v.v, vena vertebralis. (The other letters as in previous figures.)

The Pulmonary Veins

There are only two pulmonary veins, opening into the left auricle (Text-Fig. 1). The left pulmonary vein runs outwards along the precaval vein or vena anonyma for a considerable length and then turns backwards to enter the lung of its side. The right pulmonary vein, however, does not run along the vena anonyma at all It is directed backwards from the point of its opening into the heart. Both the pulmonary veins divide into two branches in the lungs.

Summary

The author gives a detailed description of the venous system of *Lissemys punctata* The more important features discovered are as follows :—

(1) The precaval vein, on each side, is composed of two tributaries—the subclavian vein and the jugular vein, but *the left jugular is a remarkably slender vessel*

(2) There are three longitudinal veins in the neck of which the middle one is the right jugular These veins are connected to each other by anastomoses.

(3) There is a *venous sinus* at the back of the head into which the cephalic veins open A detailed description of the veins of the head is given.

(4) Each kidney receives its venous blood by means of three important veins—*Vena renalis advehens anterior, Vena renalis advehens externa*, and *Vena renalis advehens posterior* All these veins are described in detail

(5) An account of the renal portal system is given. The presence of a pubic sinus and the relation of the *posterior renal advehent vein* to it are new features discovered

(6) The posterior vena cava is inclined towards the left side—a fact of phylogenetic importance The posterior vena cava is also continued behind the kidneys.

(7) The venous supply of the liver and the shell is described in detail

Acknowledgments

I am very grateful to Mr. Beni Charan Mahendra for his kind guidance and assistance during the course of the present work, as well as to the authorities of St. John's College, Agra, for the numerous facilities that I have enjoyed in the laboratory.

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A CONTRIBUTION TO THE EMBRYOLOGY OF *ENALUS ACOROIDES* (L.fil.), Steud.

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(Communicated by Dr. M. A. Sampathkumaran, M.A., Ph.D.)

Introduction

THE order Helobiales, occupying a place of special interest among the monocotyledons, has long been an extremely inviting field for morphological studies. The life-histories of a number of forms belonging to the different families of the order have been investigated and a considerable amount of literature has thereby accumulated. In this literature the family Hydrocharitaceæ, to which the plant here investigated belongs, finds a conspicuous mention.

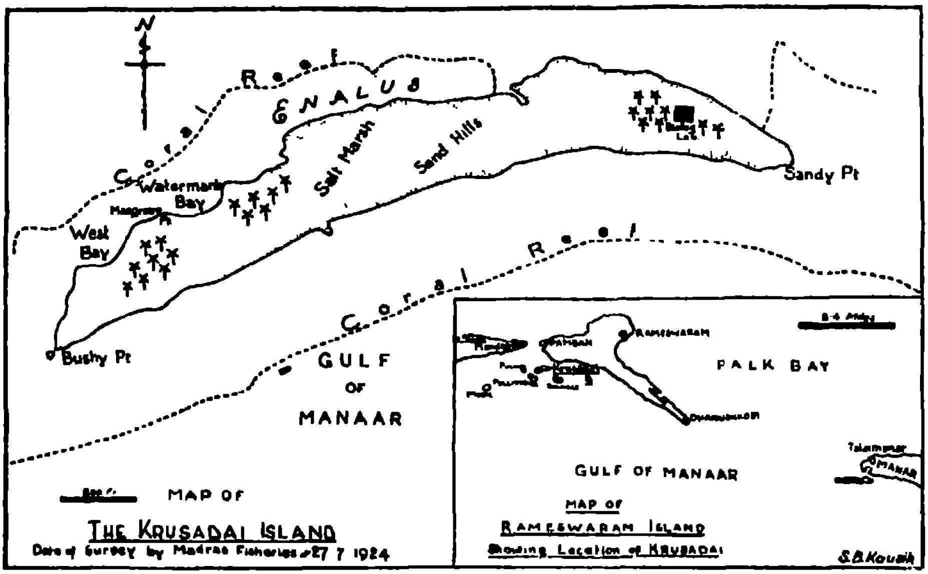
The interest in the family was first aroused by the classic work of Kerner (1891) on pollination in *Vallisneria spiralis* L. and the subsequent work of Wylie (1917) on the same aspect in the American form has added further knowledge of this most interesting phenomenon The writer has recently studied the pollination process in the case of the Indian form of *Vallisneria spiralis* L. (Kausik, 1939) and has also given a detailed account of the structural differences between this and the American form

Regarding the embryological studies in the family, Schürhoff (1926) and Schnarf (1929, 1931) have given a summary of the important contributions in their treatises on the Angiosperms so that there is no need to repeat them here. On the other hand, it is necessary to mention here only such of those embryological papers which have appeared since the publication of the works of Schürhoff and Schnarf Rangasami (1934) has studied the developmental stages in the cytology of the microspore-mother cells and the formation of the embryo-sac and embryo in the Indian form of *Vallisneria spiralis* L. A similar work in *Ottelia alismoides* has been completed by Narasimha Murthy (1935). The most recent paper in the embryology of this family is by Witmer (1937) who has made a detailed investigation of the cytology and morphology of *Vallisneria spiralis* found in America. This form is evidently *V. americana*, for Witmer (1937) himself calls attention to the fact that the American form is often known by the latter name Further, the diagnostic characters of the American form have been given by Fernald (1918) and recently Svedelius (1932) has also clearly pointed out the specific

individuality of this form It may be remarked here that while Witmer's work is the latest embryological contribution, strangely enough he does not make any reference either to the work of Rangasami (1934) on the same plant or to the paper by Narasimha Murthy (1935) on *Ottelia alismoides.*

Relating to the earlier work on *Enalus acoroides* itself there appear to be only three papers, the first of these being by Svedelius (1904) followed by the other two on the anatomy of the plant and the nature of the ovary in the pistillate flower by Cunningham (1912) and Troll (1931) respectively Svedelius (1904) deals with the general life-history of the plant treating briefly about certain stages alone in the development of the anther, the ovule and the embryo-sac; the phenomenon of pollination is especially studied in great detail The embryological features seem to be, on the other hand, only partly known and the present investigation was, therefore, undertaken

The material for the present study was collected during the month of October 1937 from plants growing on the north-western side of the island of Krusadai adjoining the larger Rameswaram island in South India (Map)



Map of Krusadai Island showing the location of *Enalus acoroides*

Both the male and the female plants grow together in large patches on the flat muddy bottom of the sea forming a kind of "submarine meadow" The plants are provided with stout rhizomes which are buried in the soil by means of numerous thick roots The leaves are coarse and strap-shaped and usually attain a length of nearly a metre. The inflorescences in the male plants and the solitary flowers in the pistillate plants are borne on well-developed stalks

or scapes The scapes increase in length to a great extent in the female plants during the development of the floral parts In connection with the staminate inflorescences in the male plants and the solitary pistillate flowers in the female plants there are found two closed spathes which are strongly keeled along the midrib to appear boat-shaped These spathes open out subsequently which is of significance, on the one hand, in loosening the innumerable staminate flowers which rise to the surface of water, and, on the other, in properly placing the pistillate flower on the surface of water for pollination

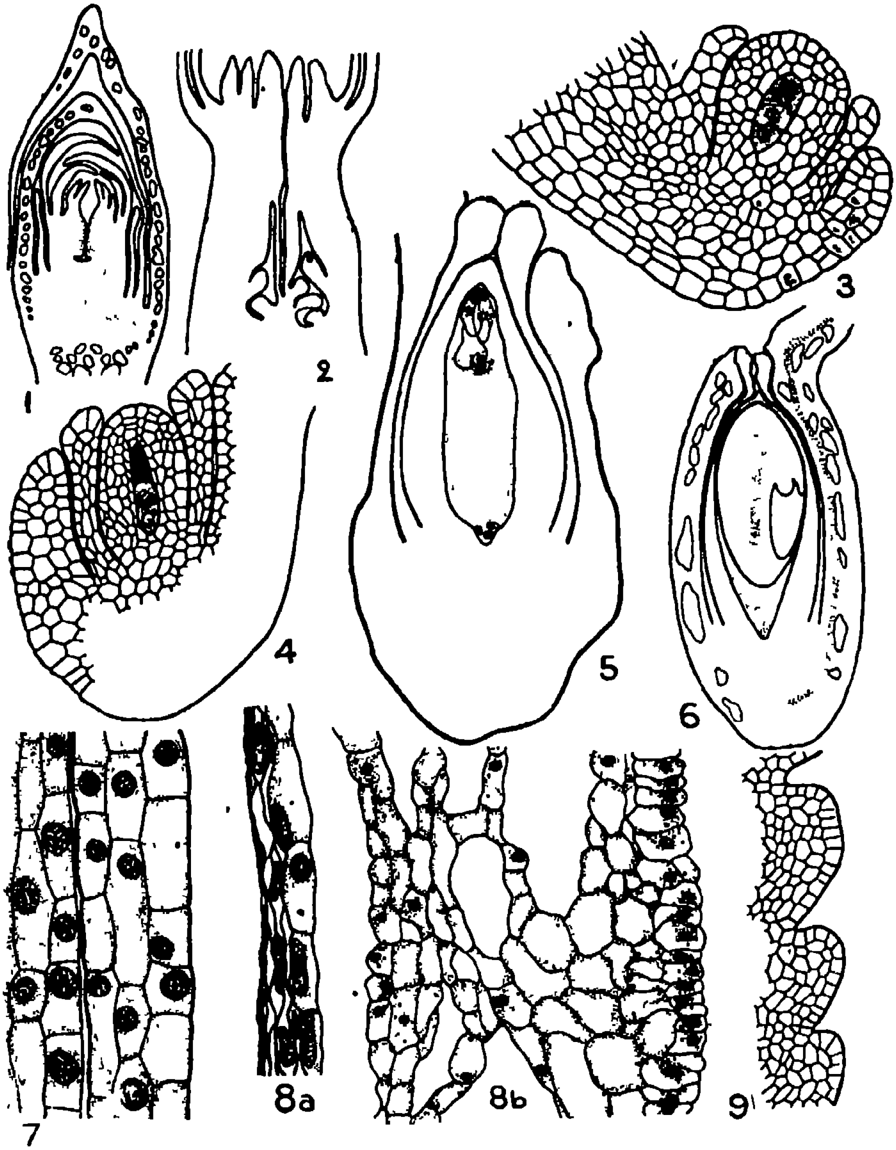
The material was collected in various stages of development of the floral parts and killed in Bouin's fluid The usual processes of dehydration and infiltration were subsequently followed and sections were cut ranging in thickness from 8μ to 14μ. Heidenhain's iron-alum hæmatoxylin was generally used for staining these sections

The Pistillate Flower

The pistillate flower develops within the closed spathes (Fig 1) and has an inferior ovary and two whorls of floral envelopes The inner floral leaves, namely the petals, are provided with a number of cross and longitudinal folds which are said to be of importance in retaining the staminate flowers during pollination (*cf* Svedelius, 1904) The ovary is made up of six carpels, the infolded margins of which protrude into the ovarian cavity as a number of paired plate-like structures These plates do not extend completely to the centre so that the ovary shows only a single large locule Svedelius (1904) regarded the paired plate-like margins of the carpels as being split, but recently Troll (1931) has clearly demonstrated that these are merely the free portions of the adjacent carpels and that the ovary in this case is really an apocarpous one He has further shown that the ovary becomes pseudo-cœnocarpous by the fusion of the outer wall of the ovary with the surrounding receptacular tissue The infolded margins of the carpels bear a few ovules which are anatropous There are a number of finger-like structures at the base of the ovary which are mucilage-secreting scales The secretion of mucilage is very evident in the pistillate flowers when they are young, but diminishes considerably as the floral parts grow older Goebel (1889–93) states that the covering of young parts with mucilage is a protection against destructive osmotic disturbances

The Ovule and the Development of the Female Gametophyte

The ovules arise as erect nucellar primordia (Figs 2 and 10) from the placenta and become anatropous gradually in the course of their development (Figs 3–5). A single hypodermal archesporial cell is soon differentiated in each primordium (Fig 10) The archesporial cell next divides once



Figs. 1-9

Fig. 1—Longitudinal section of young pistillate flower. × 20. Fig. 2—Longitudinal section of young ovary. × Approx. 27. Figs. 3 and 4—Sections of young ovules. Fig. 3 × 160; Fig. 4 × 120. Fig. 5—Section of old ovule with embryo-sac. × 80. Fig. 6—Section of seed showing the embryo. × 20. Fig. 7—Part of periphery of nucellus and inner integument to show details. × 450. Fig. 8 *a*—Part of inner integument showing the crushed cells in the seed. × 450. Fig. 8 *b*—Part of outer integument from seed to show air spaces in the tissue. × 200. Fig. 9—Part of outer surface of ovary showing the papillate projections growing out as hair-like processes. × 120.

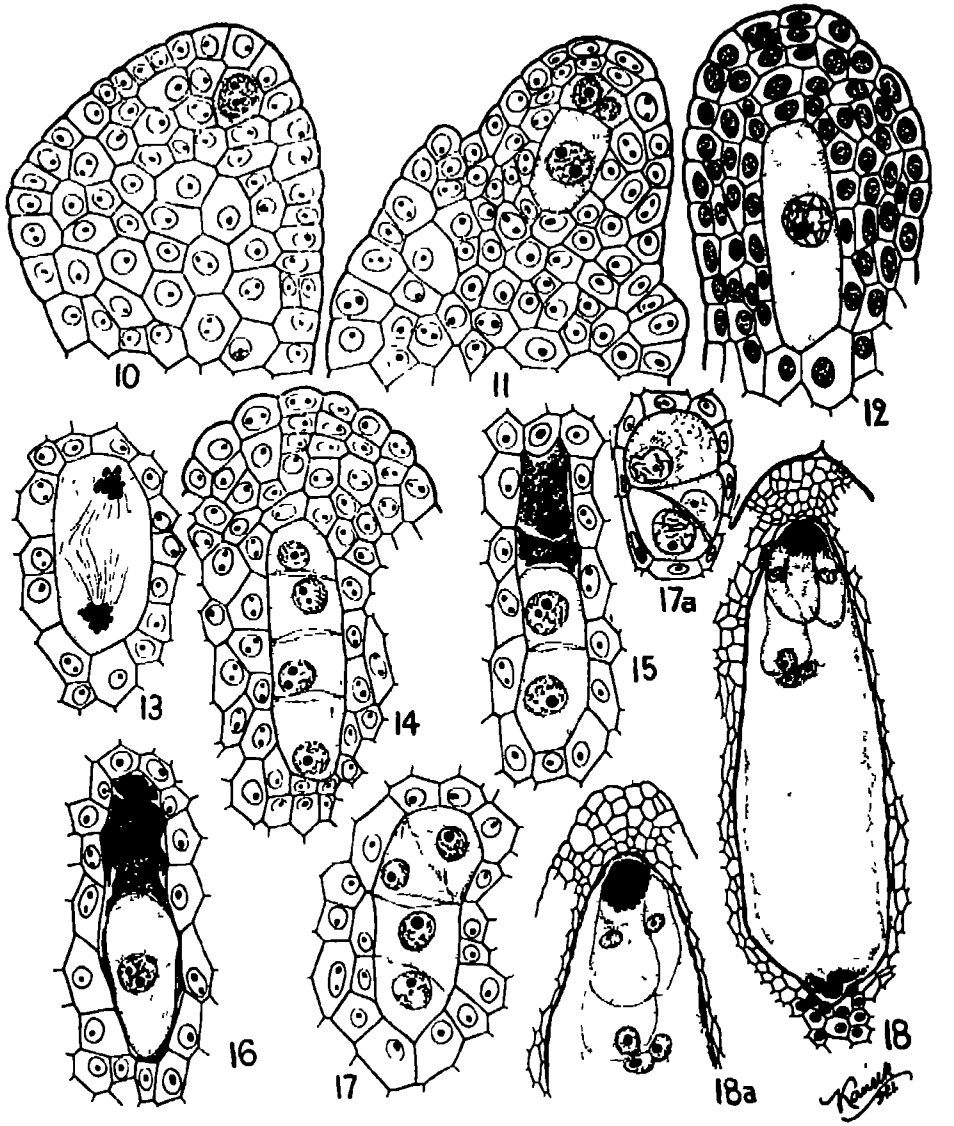
periclinally to form a primary parietal cell and the megaspore mother cell. The former immediately divides again in an anticlinal manner to form two parietal cells (Fig. 11) These two cells later undergo further divisions and give rise to a parietal tissue overlying the micropylar end of the embryo-sac.

The formation of a parietal cell is characteristic of all the Helobiales It may be mentioned here that Rangasami (1934) states that a second parietal cell is formed in *Vallisneria spiralis*, while his figure (*cf* Fig. 50) shows only a single one His statement is confusing, but in a personal talk he has now explained that he merely means that the parietal cell is second from the epidermis.

The ovule in *Enalus acoroides* shows very early during development a two-layered epidermis at the tip of the nucellus. The two layers are formed by the periclinal divisions of some of the epidermal cells at this region (Figs 12 and 14) Such an increase in the number of epidermal layers has also been shown in *Ottelia alismoides* by Narasimha Murthy (1935)

There are two integuments for each ovule and these arise as two rings all round the nucellus (Figs 3, 4 and 11). The inner integument first consists of two layers of cells, while the outer shows three layers. As the ovule grows further the inner integument becomes three-layered and the outer usually four-layered (Figs 3 and 4). There is no further increase in the number of layers in the inner integument during the subsequent stages in the development of the ovule , the inner two layers of cells become crushed later when the seed is formed The outer integument, on the other hand, shows many layers of cells during the development of the seed and a number of large air-filled cavities are also seen (Figs 7 and 8).

The megaspore mother cell enlarges considerably soon after its formation and its nucleus next enters into the meiotic divisions (Figs. 12–14) in the formation of the linear tetrad of megaspores. Sometimes the tetrad shows a T-shaped arrangement on account of the disposition of the upper two megaspores in an oblique manner (Fig. 17) In very rare cases the megaspores formed by the upper and lower dyad cells during the second division were also met with in such a manner that they were disposed at different intersecting angles to the median plane of the ovule. In these cases, a tetrahedral arrangement would result (Fig 17 *a*). In this connection it is interesting to note that in another member of the Hydrocharitaceæ, namely *Vallisneria spiralis*, Witmer (1937) has observed tetrahedral arrangement of the megaspores in addition to the linear and T-shaped tetrads Maheshwari (1937) states that a tetrahedral arrangement was noted in recent literature in *Nymphoides peltatum* (Stover, 1932) only The observations made by



FIGS. 10-18

Fig. 10—Young ovule with the hypodermal archesporial cell. × 400. Fig. 11—The megaspore mother cell and the two parietal cells. × 400. Fig. 12—The enlarging megaspore mother cell with nucleus in spireme. × 400. Fig. 13—First division (telophase) in megaspore mother cell. × 450. Fig 14—Linear tetrad of megaspores. × 900. Fig. 15—The upper two megaspores degenerating. × 450. Fig. 16—All the three upper megaspores degenerating and the lowest alone surviving. × 400. Fig. 17—The spindle for the upper two megaspores oblique. × 450. Fig. 17 *a*—Tetrahedral arrangement of megaspores. × 450. Fig. 18—The fully developed embryo-sac. × 160. Fig. 18 *a*—The egg-apparatus enlarged. × 200.

Witmer (1937) and the writer would then add two further instances of this to the literature.

After the tetrad of megaspores is established the upper three begin to degenerate (Fig 16). The degeneration begins first in the upper two megaspores while the third is yet quite intact (Fig. 15) Finally, the latter also degenerates and the chalazal megaspore alone remains intact This enlarges further (Fig. 16) and develops into the embryo-sac. The course of development of the embryo-sac follows the normal type. The fully formed embryo-sac is very large with a large micropylar end and a bluntly tapering pouched antipodal end (Fig 18)

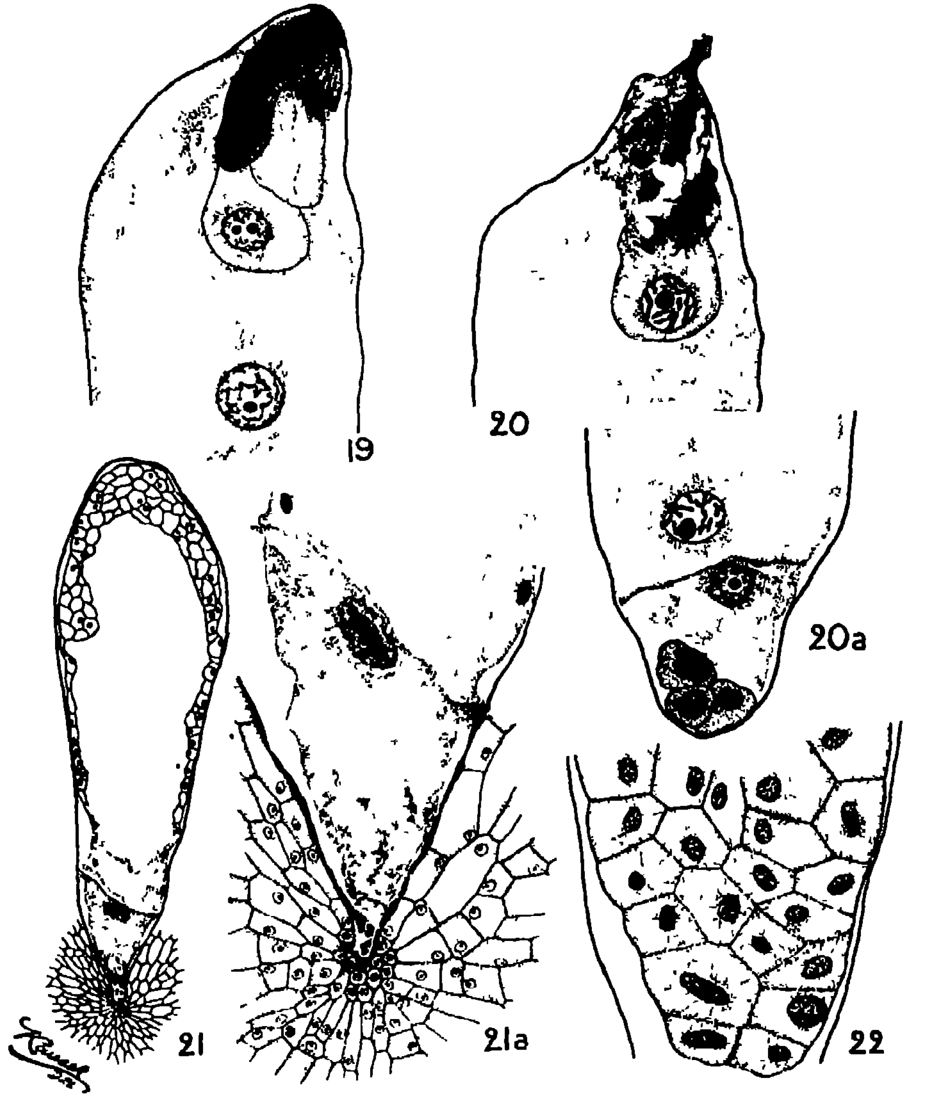
The micropylar end of the embryo-sac contains the egg-apparatus showing the two synergids and the egg (Fig. 18 *a*) The synergids are long and sac-like with a large basal vacuole in each The tips of the synergids contain dense cytoplasm and develop into a well-defined *filiform apparatus* The egg is very much longer than the synergids and its base extends far beyond them In close contact with the egg there are two polar nuclei which are pressed against each other (Fig 18 *a*) While actual fusion of the polar nuclei was not seen, there is strong ground to infer that fusion takes place before fertilization, for a single larger nucleus was seen in their place in some embryo-sacs into which the pollen tubes had entered but had not as yet discharged their contents (Fig 19)

The pouch-like antipodal end of the embryo-sac contains three large antipodal cells (Fig 18) This end forms after fertilization a very much tapering region of the embryo-sac, which seems to be connected with a nutritive function and the nucellar cells show a regular radiating arrangement at this region This point has also been noted by Svedelius (1904)

The fully formed embryo-sac contains a large cavity in the centre, the cytoplasm being restricted only to the two poles and as a thin layer along the sides (Fig 19) Many of the adjacent cells of the nucellus appear very much crushed and their cell outlines rather irregular during the formation of the embryo-sac

Fertilization

In spite of collecting the material in all available stages of development, both prior to and after pollination, stages showing actual fertilization were not seen In a few preparations, however, the pollen tubes were noticed inside the embryo-sacs, but in these preparations the tubes were either quite intact immediately preceding the dischaige of contents (Fig. 19) or were empty (Fig 20) and in the latter case fertilization was over In all these cases the pollen tubes were densely stained and, therefore, even in those in



FIGS. 19-22

Fig 19—Micropylar portion of embryo-sac with the pollen tube intact. The polar nuclei have fused. × 400. Fig. 20—Micropylar end of embryo-sac soon after fertilization. The egg nucleus is preparing to divide. × 400. The antipodal end of same showing the separation of the chalazal chamber as the *basalapparat* by the formation of a wall between the endosperm nuclei formed after the first division of the primary nucleus. × 400. Fig. 21—The embryo-sac showing the cellular endosperm in the upper primary micropylar chamber and the lower primary chalazal chamber (the *basalapparat*) containing a large densely staining nucleus. Note the radiating cells of the nucellus from the antipodal end of the sac both in this and in the next figure. × 40. Fig. 21 *a*—Lower portion enlarged from Fig. 20. × 120. Fig. 22—Tangential section of endosperm (portion only) showing wall-formation which is simultaneous, × 200.

which the contents were not discharged it was not possible to make out the nature of the male nuclei. It should be interesting to study, at some future time when sufficient material showing these stages could be gathered, the exact nature and behaviour of the nuclei during syngamy and double fertilization with particular reference to the interesting observations of Wylie (1923) on the male cells of *Vallisneria spiralis.* Cooper (1936) has recently shown that the cytoplasm of the male nucleus is retained until the time of actual fusion in *Lilium.*

The Embryo

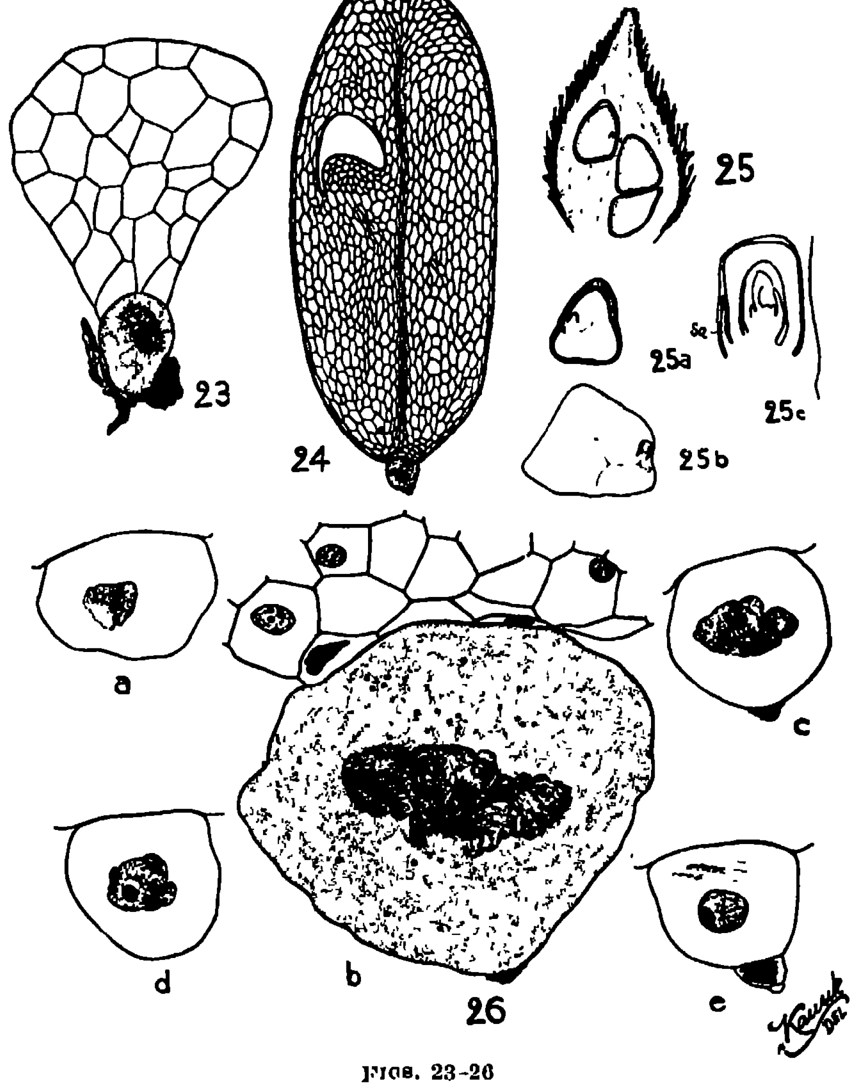
The first division of the zygote nucleus seems to take place only after the primary endosperm nucleus has divided; it was noticed that the zygote nucleus was still in late prophase preparing to divide, while at the lower end of the embryo-sac where the first division of the primary endosperm nucleus takes place, two large nuclei were seen separated by a transverse wall (Figs. 20 and 20 *a*). The course of development of the embryo is presumably similar to that in the other members of the family During the development of the embryo a large basal cell is conspicuously seen and this cell persists till a very late stage in the seed (Figs 23 and 24). The basal cell seems to take part in absorbing nutrition for the growing embryo and its nucleus, staining very intensely, is evidently associated with this to a large extent. When the embryo reaches maturity the activity of the basal cell declines and its nucleus becomes highly hypertrophied and irregular in contour with a number of lobes all over (Fig. 26 *a* to *e* showing the basal cell in a complete series of sections) Finally the basal cell collapses and the nucleus degenerates.

A large basal cell is seen in many other members of the family, for example *Elodea* (Wylie, 1904), *Vallisneria spiralis* (Rangasami, 1934 ; Witmer, 1937) and *Ottelia alismoides* (Narasimha Murthy, 1935)

The fully developed embryo of *Enalus* which occupies completely the cavity of the seed (Fig 6) after destroying the endosperm, is typically monocotyledonous. The stem tip develops laterally inside a depression at the base of the large and massive cotyledon The cells of the cotyledon are abundantly filled with starch grains The radicle is blunt and broadly conical. The basal cell may be recognized at the tip of the radicle even in late stages.

The Endosperm

As already stated the primary endosperm nucleus divides earlier than the zygote nucleus. This division takes place at the lower end of the embryo-sac and is followed by a transverse wall (Fig 20 *a*). Two chambers are thus



Figs. 23-26

Fig. 23—Young embryo with a large basal cell. × 160. Fig. 24—Old embryo still showing the large basal cell, the terminal cotyledon with central plerome strand, the radicle and the stem tip appearing laterally in a notch. × 40. Fig. 25—Longitudinal section of fruit × ½. Fig. 25 *a*—Longitudinal section of seed. × ½. Fig. 25 *b*—Same enlarged to show massive cotyledon, plumule, the radicle which does not function and the origin of a second root laterally at the base of the plumule. × 1. Fig. 25 *c*— The plumule enlarged to show the succession of leaves and the presence of the scales, the *squamulæ*, sq. Fig. 26 *a-e*—The basal cell of embryo in Fig. 24 followed through all sections to show the formation of lobes from the nucleus. All × 200 except *b* × 450.

formed in the embryo-sac, the upper larger primary micropylar chamber and the lower smaller primary chalazal chamber The nucleus of the former undergoes further repeated divisions to form a number of free nuclei which become arranged along the periphery of the embryo-sac. The nucleus in the chalazal chamber, on the other hand, remains without dividing any further The development of endosperm thus conforms to the type characterizing the order Helobiales

The free nuclei formed in the primary chalazal chamber begin to organize themselves as cells very soon When this cell organization begins the embryo is a small mass of cells without any differentiation and lies at the micropylar end The cell formation in the micropylar chamber is simultaneous at particular regions of the embryo-sac (Fig 22) Cell formation is entirely suppressed in the centre of the embryo-sac which contains only a sap-like fluid Cell formation is very clear only at the two poles and along the sides as a thin layer. The endosperm is completely used up by the embryo and the mature seed, therefore, becomes non-endospermic

The primary chalazal chamber where the single nucleus remains without dividing any further constitutes a haustorial organ of the nature of the *basalapparat* It shows dense cytoplasm and a darkly staining nucleus The nucellar cells at this region radiate regularly and contain rich cytoplasm. These cells seem, therefore, to be related with a nutritive function. In the developing seed the activity of the chalazal chamber gradually declines and its nucleus becomes hypertrophied and irregular Still later it undergoes actual fragmentation and becomes resolved into a number of darkly staining granules of varying sizes scattered in the cytoplasm In later stages the *basalapparat* disappears completely and only a large cavity is left over in its place Prior to the decline of the *basalapparat* the three antipodal cells may be seen as darkly stained bodies at the lower end of the embryo-sac (Fig 21*a*)

The Seed

The fruit of *Enalus acoroides* develops suspended in sea-water at varying levels below the surface The outer surface of the fruit is densely clothed over by numerous stiff bristles and the tissue within is soft and fleshy Embedded within the soft tissue are a few large seeds placed loosely within cavities When the fruit dehisces the seeds are exposed and come in direct contact with the surrounding sea-water Since the outer integument has numerous air-filled cavities the buoyancy of the seed is increased and so the seed does not immediately sink in water Later, however, the seed-coat comes off as a loose cap by becoming ruptured at the base and the embryo thus becomes naked

This naked embryo is very massive and sufficently heavy to drop directly into the soft soil. It is conical in shape (Fig. 25 *a* and *b*) with a large cotyledon containing plenty of starch-filled cells. The radicle occupies the flat portion of the embryo and is non-functional in subsequent stages. The plumule is well developed and shows a number of young leaves arising in quick succession (Fig 25 *b* and *c*) At the base of the plumule and a little to the side is formed a second root which later comes out during the germination of the embryo in the soil.

In connection with the young leaves of the plumule there are certain axillary scale-like structures (Fig. 25 *c*) These are the well-known *squamulæ* which are said to be characteristically associated with the leaf-bases in the Helobiales by Arber (1925)

The heavy embryos of *Enalus* which leave the seed-coat sink in the sea-water immediately They become buried in the soil where they at once begin to grow further. Svedelius (1904) calls attention to the characteristic germination of the embryo and remarks: "*Enalus acoroides*....belongs to that group of plants where the young embryos or spores develop directly without any period of rest. Goebel especially has shown that this phenomenon is not rare in plants growing in wet localities, and that the well-known 'vivipary' in the Rhizophoraceæ and the other mangrove plants may be in some respects regarded as a special case of this rather common general phenomenon." He further states. "One of the characteristics of *Enalus* is that the young embryos are dispersed directly, the plant having no seeds. These embryos, however, have not reached so high a degree of development in connection with the mother plant as in the Rhizophoraceæ, nor are they provided with any endosperm But the cotyledon is plentifully provided with food, and the further development can go on directly, because the plant lives under conditions in tropical sea where the period of vegetation is never interrupted by external influences"

Conclusions

The significant features in the life-history having been pointed out in the above account, a comparison may now be made between *Enalus acoroides* and the other members of the Hydrocharitaceæ. In the development of the ovule and in the formation of the megaspores and the embryo-sac no essential departures are met with The usual variations in the arrangement of the tetrad of megaspores are noticed and rarely a tetrahedral arrangement is also seen as in *Vallisneria spiralis* (Witmer, 1937).

In *Ottelia alismoides* it is stated by Narasimha Murthy (1935) that a parietal cell is not found. He further comments that while the parietal cell

formation is considered to be a general feature in the family by Schürhoff (1926) *Ottelia alismoides* and *Vallisneria spiralis* (according to Rangasami, 1934) resemble each other in the absence of a parietal cell On the other hand, Rangasami (1934) clearly states that the megaspore-mother cell is formed after the parietal cell is cut off by the archesporium. His figure (*cf* Fig 50) also shows this point clearly. As already stated his statement is rather ambiguous, for he refers to a " second parietal cell" By this he only means (as explained personally by him) that the parietal cell is second from the epidermis. A single parietal cell is also noticed by Witmer (1937) in the American *Vallisneria*

From the above considerations it may be remarked here that it is highly probable that a parietal cell is present in *Ottelia alismoides* also. A more critical examination of the young ovule at this stage should have made this point clearer Narasimha Murthy's figures of older ovules show a mass of parietal tissue overlying the embryo-sac and this tissue is probably formed by the divisions of a primary parietal cell as in most cases Narasimha Murthy, however, regards this tissue to have wholly arisen on account of the divisions of the epidermal cells of the nucellus In *Enalus acoroides* the epidermal cells also divide in addition to the formation of a parietal tissue by a primary parietal cell.

The formation of the linear tetrad of megaspores and the subsequent behaviour of the chalazal megaspore to form a typical eight-nucleate embryo-sac in *Enalus acoroides* are very similar to those in the other investigated members of the family that a further detailed discussion is unnecessary But it is significant to note that the antipodal end of the embryo-sac which is pointed and narrow to form a deep pouch after fertilization, is concerned to a great extent in absorbing nutrition from the chalazal cells The latter contain rich cytoplasm and are found in regularly arranged radiating rows Svedelius (1904) also suggests a nutritive function to this structural feature Wylie (1904) has described the formation of an antipodal pouch in *Elodea canadensis* and remarks: " While such a pouch-like antipodal end is not uncommon, the emphasis laid on its early development in this instance (*Elodea canadensis*) might suggest its being a rudiment of a once prominent nutritive device, but it probably functions now in no important way." In the case of *Enalus acoroides*, however, the great development of the antipodal end after fertilization and its association with radiating cells of the nucellus is a strong indication of its being a haustorial organ.

The disposition and the nature of the egg apparatus and the polar nuclei in very close proximity, as also the general configuration of the embryo-sac

itself, are very similar to the conditions met with in *Elodea canadensis* (Wylie, 1904) and *Vallisneria spiralis* (Rangasami, 1934 ; Witmer, 1937) A further resemblance between *Elodea* and *Enalus* may be seen in the behaviour of the polar nuclei, which fuse only just prior to fertilization in both The two nuclei remain in close contact with each other for a considerable time before actually fusing The entry of the pollen tube may offer some stimulus in effecting their fusion In the case of *Vallisneria spiralis* the fusion is effected frequently even before the pollen tubes enter the embryo-sac according to Witmer (1937)

A prominent basal cell for the embryo has been shown in most of the Hydrocharitaceæ, as in *Elodea* (Wylie, 1904), *Ottelia* (Palm, 1915 , Narasimha Murthy, 1935) and *Vallisneria* (Rangasami, 1934 ; Witmer, 1937) In all these, as also in *Enalus acoroides*, it persists even in the very old embryos The presence of an intensely staining large nucleus in the basal cell in *Enalus* is strongly suggestive of a nutritive function Finally when the basal cell collapses, the nucleus becomes hypertrophied and breaks down

The development of the endosperm tissue in the upper primary micropylar chamber of the embryo-sac and the separation of the lower smaller chalazal chamber of the nature of a *basalapparat* are common features in the order Helobiales A further discussion is, therefore, unnecessary here A minor comment however seems inevitable in view of the fact that in another member of the family, *Ottelia alismoides*, Narasimha Murthy (1935) remarks that the chalazal chamber, though at first small, later grows enormously and equals in size the primary micropylar chamber This appears to be certainly striking, but the figures which he gives in support do not seem to suggest in any way such a behaviour of the chalazal chamber The chalazal chamber, as seen from the figures, appears to be quite similar to the same in the other members of the Hydrocharitaceæ and is also small in proportion to the rest of the embryo-sac

In concluding this study of the embryology of *Enalus acoroides*, it may be well to emphasize that the plants are so well adapted to their surroundings in the sea, where the current of water may often reach a speed of as much as 5 to 6 knots in an hour (Walther, 1891), that there are found a number of structural features which play an interesting rôle in the life history of the plant The petals are long and delicate with a number of folds They are thus admirably suited for catching and retaining the staminate flowers for pollination even when considerable disturbances are present on the surface of water Further, the stages in pollen transfer are definitely correlated with the tidal movements of the sea as suggested by Svedelius (1904) In this

respect, *Enalus acoroides* offers a case as interesting as *Vallisneria spiralis* (Kerner, 1891, Wylie, 1917, Kausik, 1939) With the accomplishment of pollination, which is the preliminary phase in the final production of fruit under the surface of water, the seeds begin to develop under very uniform conditions in the tropical sea, the embryo becomes well suited, by its peculiar characteristics, for immediate and ready germination in the soft soil A distinct type of vivipary is exhibited by the plant

Summary

The ovules are anatropous and have two integuments The inner integument becomes crushed during the development of the seed, while the outer becomes thick and shows a number of large air-filled cavities

The young ovule primordium shows a single hypodermal archesporial cell which divides to form a parietal cell and the megaspore-mother cell The former gives rise to a parietal tissue In the meanwhile, the epidermal cells at the tip of the nucellus also divide once periclinally to form two layers.

The megaspore mother cell undergoes the usual two divisions to form a linear tetrad of megaspores In addition to the typical linear arrangement, a T-shaped tetrad and very rarely a tetrahedral arrangement are also seen

The chalazal megaspore gives rise to a normal eight-nucleate embryo-sac The fully formed embryo-sac is extremely large with an immense vacuole in the centre. The synergids are sac-like and their tips show a well-defined *filiform apparatus* The egg is longer than the synergids and the two polar nuclei lie in close contact with each other in proximity with the egg apparatus. The fusion of the polar nuclei is delayed and takes palce only before fertilization

The lower end of the embryo-sac contains three antipodal cells This end becomes conspicuous as a deep pouch after fertilization and in association with regularly arranged rows of cells of the chalazal region takes part in the absorption of nutrition

The zygote nucleus undergoes the first division followed by a transverse wall only after the primary endosperm nucleus has divided once at the lower end of the embryo-sac The further development of the embryo is similar to that in the other members of the family A large basal cell is seen developing prominently in the embryo This basal cell has a large nucleus, which later becomes hypertrophied and forms a number of peripheral lobes Finally the basal cell collapses The fully developed embryo shows a well-formed

plumule and a laterally arising secondary root which functions during germination.

The endsoperm is of the *Helobiales*-type. The primary chalazal chamber forms a haustorial organ of the nature of the *basalapparat*.

The mature seed is non-endospermic Its structure is pointed out in the paper The phenomenon of vivipary which is exhibited by *Enalus acoroides* is discussed in the paper.

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AN EMBRYOLOGICAL STUDY OF *SURIANA MARITIMA* LINN.

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Introduction

Suriana maritima L belonging to the Simarubaceæ is a small shrub distributed along the tropical coasts The species has recently been investigated by Wiger (1935) who has given an account of the endosperm and the embryo in his comparative embryological study of the Simarubaceæ It is therefore, proposed to give here in detail an account of the aspects which have not been mentioned by Wiger (1935)

Previous to the work of Wiger (1935) the only other reference to the embryological literature in the family is an account of the life history of *Ailanthus altissima* by Schürhoff (1924) (Schnarf, 1931)

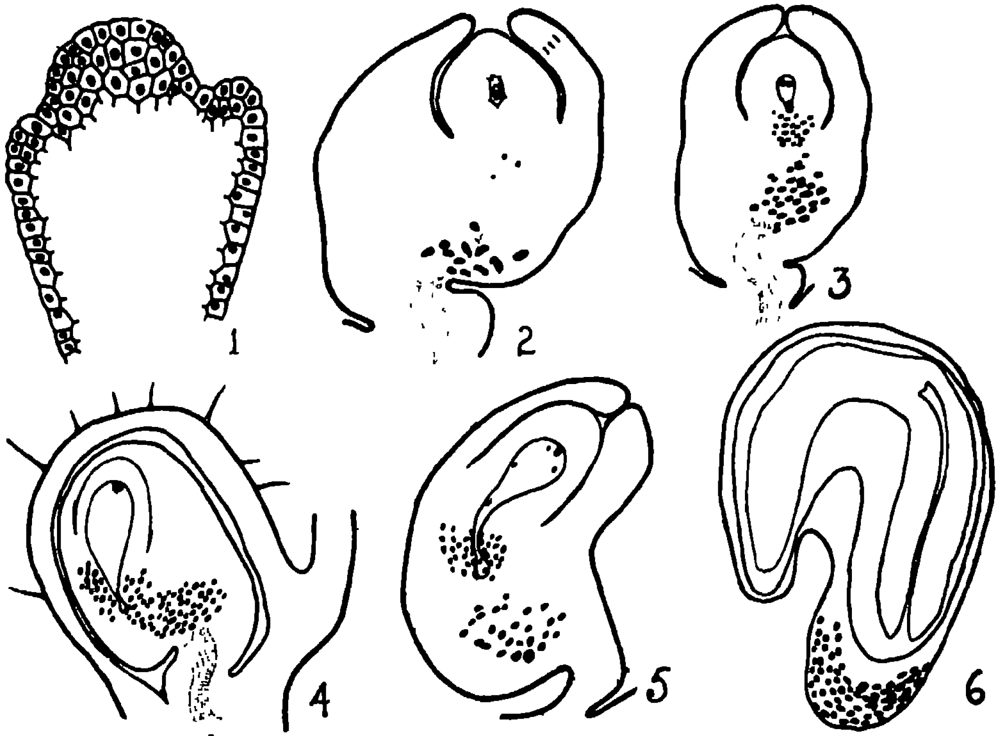
Material and Methods

The material for study was collected at Krusadai and Shingle islands, two very small islands adjoining the Rameswaram Island, S India A few stray plants are found in these islands Collections were made during the month of October and the material was fixed in Bouin's fluid Material fixed in formalin-acetic-alcohol was also made use of Sections were cut at thicknesses varying from 6 to 12 microns and stained in Heidenhain's iron-alum-hæmatoxylin

Observations

The flowers are produced freely during the months, September and October The hermaphrodite flowers are borne terminally and are usually covered over by the leaves The ovary and the sepals are lined with multicellular hairs The fruit might consist of all the five carpels or as is often the case of fewer carpels. These are covered by the persistent calyx.

A characteristic feature is the presence of a substance which takes a dense and homogeneous stain in many cells of the floral parts particularly in the epidermal cells



FIGS. 1-6. Stages in the development of the ovule

Fig. 1—The nucellus and appearance of the single integument. × 450. Figs. 2-5—Ovule at various stages showing the gradual curving and the presence of the hypostase. Figs. 2 and 3 × 200; Figs. 4 and 5 × 80. Fig. 6—Late stage showing the ovule completely bent and enclosing the curved embryo. × 10.

The ovule presents certain interesting features during the various stages of its development It is first evident as a nucellar mass and the integument initial also appears (Fig 1). At this stage only a few cells of the nucellus at the base of the ovule contain dark staining substances The integument soon covers the nucellar epidermis and the hypostase can be seen at the base of the ovule (Fig 2) The vascular supply to the ovule ends just beneath the hypostase. In later stages the hypostase is well developed and remains conspicuous even in the mature seed (Figs 3, 4, 5, 6) In addition to this, most of the cells of the chalaza surrounding the lower half of the embryo-sac possess very dark staining substances (Figs 11, 13)

During the post-fertilisation stages the ovule undergoes a remarkable curvature due to extensive differential growth, resulting in the ovule becoming completely campylotropous These changes have already been described in detail by Wiger (1935).

The single integument grows rapidly and completely covers the nucellus. The archesporial cell is not easily evident in the hypodermal layer of the nucellus The archesporial cell is recognised only after a few parietal cells are formed so that the megaspore-mother cell is situated deep in the nucellus (Fig. 7) Wiger (1935) referring to *Samadera* species says, " Neither in this nor in other species of this family can one speak of a marked subarchesporial cell." The epidermis of the nucellus also divides so that the megaspore-mother cell comes to lie about five or six cells deep in the nucellus

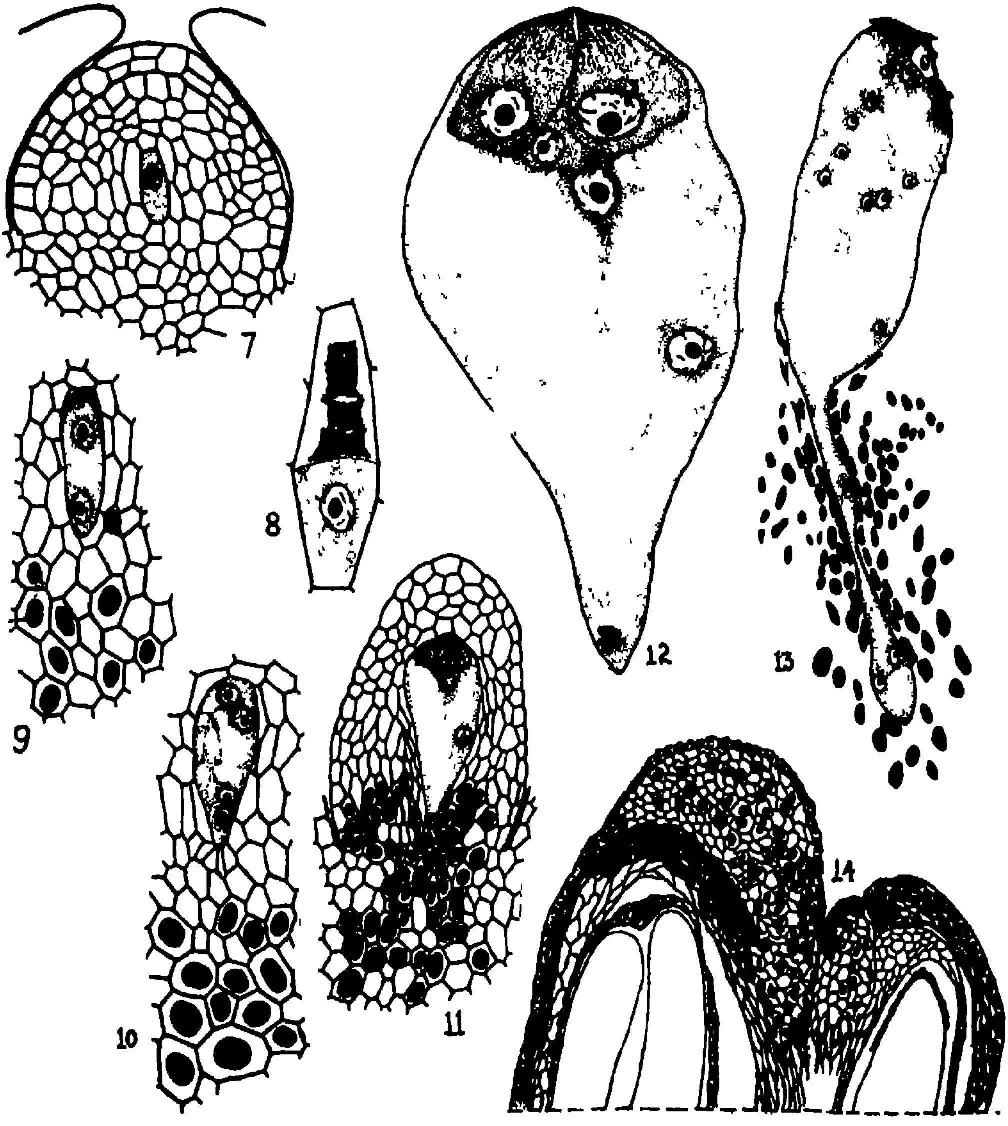
The megaspore-mother cell elongates and undergoes the usual two divisions giving rise to the linear tetrad of megaspores. In all the species investigated by Wiger (1935) with the exception of a solitary case of a T-tetrad in *Ailanthus malabaricum* no other arrangement other than that of a row tetrad has been noticed. In *Suriana maritima* the chalazal megaspore is found to develop further (Fig. 8). The chalazal megaspore enlarges and a division of the nucleus results in the two nucleate embryo-sac A large vacuole is formed in the centre The remains of the degenerated megaspores can be seen as a darkly staining cap above the embryo-sac (Fig 9) The next nuclear division in the embryo-sac results in the four-nucleate sac. The embryo-sac at this stage is observed to have a narrow antipodal end (Fig 10).

The embryo-sac grows considerably during the further stages and the mature embryo-sac is nearly thrice the size of the four-nucleate one. The fully formed embryo-sac has a broad micropylar end and a very narrow chalazal end which is wedged between the chalazal cells (Fig 11)

In the fully formed embryo-sac which is of the normal type the egg apparatus consisting of the two synergids and the egg, the two polar nuclei and three antipodals are seen. The antipodals, however, degenerate early and so at maturity only the remains of the antipodals are seen The antipodals are found to be ephemeral in all the species investigated by Wiger (1935)

The synergids are broad and the nuclei are found rather low down so that no basal vacuole is seen Later, however, the basal vacuoles are observed. At the tip of the synergids a few thread-like structures constituting the filiform apparatus are present This structure, however, is not prominent Such a " pointstructure " has been noticed in some species by Wiger (1935)

Though actual stages in fertilisation were not available in the material examined, it can safely be assumed that normal fertilisation takes place because the remains of the pollen tube were seen in many ovules.

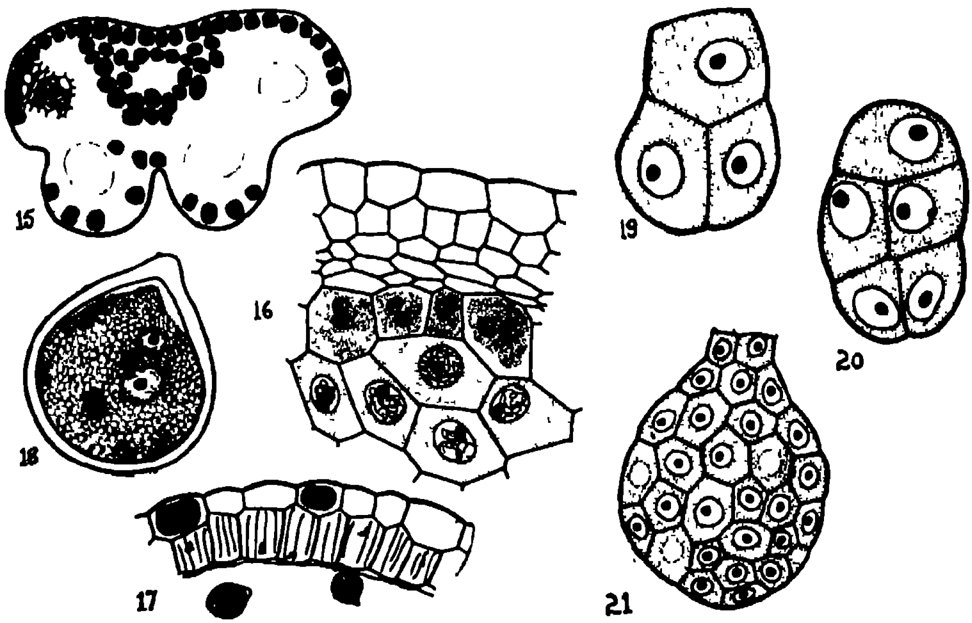


FIGS 7-14

Fig. 7—Young ovule with the deep-seated megaspore-mother cell. × 450. Fig. 8—Linear tetrad of megaspores. The upper three megaspores have degenerated and the lowermost is enlarging. × 1350. Figs. 9 and 10—Two and four-nucleate embryo sacs. × 630. Fig. 11—Fully formed embryo-sac and the nucellar cells at the chalaza containing the dark staining substance. × 270. Fig 12—Organised embryo-sac with the degenerated antipodals. × 900. Fig. 13—Post-fertilisation stage showing the elongated antipodal end of the embryo-sac. × 200. Fig. 14—Section of a portion of late ovule showing the micropyle and the chalaza side by side. × 80.

During the post-fertilisation stages the antipodal end of the embryo-sac grows in length in a remarkable manner It pierces through the chalazal cells as a slender tube and is bulged at the tip. A few endosperm nuclei are present at this tip. The number of nuclei gradually increases and cell-formation begins at the micropylar end. With the accompanying curving of the ovule itself the embryo-sac also undergoes the curvature. These changes have been described by Wiger (1935). It may only be added here that in the seed the micropyle and the chalaza are side by side and in contact (Fig 14) The nucellus is intact at the micropyle but is crushed at the sides The cells of the integument are highly compressed and take a deep stain The endosperm is present as a thin layer at certain places along the embryo The endosperm becomes cellular even at the chalazal end in the final stages.

The fertilised egg divides after a number of free endosperm nuclei are formed. The first wall is transverse resulting in the apical and the basal



FIGS. 15-21

Figs. 15-18—The anther and formation of pollen. Fig. 15—Transverse section of young anther. × 200. Fig. 16.—Portion of young anther wall and the sporogenous cells. × 900. Fig. 17—Old anther wall with the endothecium having the fibrillar thickenings. × 200. Fig. 18—Pollen grain at time of shedding. × 900. Figs. 19–21—Stages in the development of the embryo. Figs. 19 and 20 × 1350. Fig. 21 × 600.

cells. In the former a vertical division takes place (Fig. 19), followed by a transverse division in the basal cell The next division in the middle cell is vertical and this results in the five-celled embryo (Fig 20) Thereafter no regular divisions seem to take place and a large spherical embryo is formed (Fig. 21). The late embryo is curved and occupies a large part of the seed

A detailed study of the development of the anther and the meiosis of the microspore-mother cells could not be made for want of material A cross-section of the anther shows the four-lobed nature. The epidermal cells contain the deeply staining substances as also the cells around the vascular trace (Fig 15) The wall of the young anther shows the epidermis, the endothecium, two or three middle layers and the tapetum The epidermal cells have the dark staining substances (Fig 16) The tapetum is uninucleate at the beginning but later when the microspore-mother cells are in synapsis, the nucleus of the tapetal cell undergoes a division The tapetal cells thereafter remain binucleate (Fig 16) In the ripe anther, the endothecium develops the fibrillar thickenings (Fig 17) As has been noted in other Simarubaceæ the pollen grain is two-nucleate at the time of shedding (Fig 18)

Conclusions

As stated by Wiger (1935), only *Suriana maritima* among the investigated species of the Simarubaceæ, has a single integument No traces of the second integument are visible at any stage The nucellus is massive and a parietal tissue is formed The cap tissue, however, is not prominent

The embryo-sac undergoes extensive growth in two distinct stages. The first is during the formation of the eight-nucleate sac from the four-nucleate one The second stage in the growth of the embryo-sac is the post-fertilisation one The antipodal end elongates considerably into a haustorial organ Further growth of the embryo-sac results in its becoming completely curved

The presence of the hypostase has been observed in some of the species investigated by Wiger (1935) In *Suriana maritima,* in addition to the hypostase, most of the cells surrounding the lower half of the embryo-sac possess a substance which takes an intense black stain The hypostase has been noticed in various plants by previous investigators. It seems to be a constant feature of the Onagraceæ. According to Johansen (1928) "it is a flexible adaptation to the environment that makes its appearance only when necessary and serves to stabilise the water balance of the resting seed over the long period of dormancy during the hot seson". This statement may, well be true of *Suriana maritima* as it is one of the tropical sea-shore plants

where there is a likelihood of such dry conditions existing. The presence of the dark staining substance in many cells of all the parts of the flower which is such a marked feature in *Suriana maritima* may also be a result of its habitat

Summary

The ovule possesses a single integument The megaspore-mother cell is deep-seated on account of the development of the parietal tissue A linear tetrad of megaspores is produced and the lowermost megaspore produces the embryo-sac

The development of the embryo-sac is normal The fully formed embryo-sac has a broad micropylar end and a narrow chalazal end The antipodals degenerate early The nucellar cells around the antipodal end of the embryo-sac are seen to possess a dark staining substance

The antipodal end of the embryo-sac grows considerably in length during the post-fertilisation stages and forms a long tubular structure. The ovule undergoes a curvature and becomes campylotropous in later stages, enclosing the curved embryo

A well-defined hypostase is present in the ovule

The pollen grain at the time of shedding is two-nucleate

The writer wishes to express his gratitude to Dr. M A. Sampathkumaran, Head of the Department of Botany, University of Mysore, for guidance and kind encouragement Sincere thanks are due to Mr S B Kausik for kindly collecting some of the material used in this study

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EMBRYOLOGICAL STUDIES OF SOME MEMBERS OF RHAMNACEÆ*

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Received January 23, 1940

(Communicated by Prof. M. A. Sampathkumaran, M.A., Ph.D.)

The Rhamnaceæ, consisting of 40 genera and 500 species, are cosmopolitan and very common in the wild condition (Willis, 1931). Most of them are either trees or shrubs possessing thorns and some, like *Ventilago*, climb by means of tendrils. The fruits of various species of *Rhamnus* yield dyestuffs and those of *Zizyphus* are edible. The wood of many give good charcoal and the roots of some are of medicinal value.

The earliest scientific work in this family is that of Ward (1887) who worked on the histology and physiology of the fruits of *Rhamnus*. Lindau (1928) has reported the occurrence of perisperm in some species of *Rhamnus* and he has also studied the development of the integuments in *Rhamnus cathartica*. The only recent work is that of Chiarugi (1930) on *Zizyphus sativa*, in which he reports the "Scilla-type" of development for the female gametophyte and records the haploid number of chromosomes to be 13. Schnarf (1926) and Schnarf (1931) have summarised the work done in this family.

The present study was undertaken to investigate the life-histories of *Zizyphus Jujuba* Juss., *Zizyphus Œnoplia* Mill. and *Scutia myrtina* Kurz. Particular attention is paid to the development of the female gametophyte and the embryo.

Materials and Methods

The materials from *Zizyphus Jujuba* were collected in October and November while those of *Zizyphus Œnoplia* and *Scutia myrtina* were collected in March, April and May, from plants growing in and around Bangalore. They gave very satisfactory results when fixed between 11 A.M. and 2 P.M. on warm sunny days. The materials fixed in various combinations of acetic alcohol did not cut well as they were hardened by the fixative. Nawaschin's fixative gave fairly good results for *Scutia myrtina*. Of all the fixatives tried,

* Part of thesis approved for the degree of Master of Science in the University of Mysore, 1938.

by far the best results were obtained with Bouin's killing fluid and Allen's modified Bouin Much trouble was experienced in cutting as all the floral parts are coated with dense hairs and contain plenty of tannin in their cells but the difficulty was overcome by removing these parts before fixing. Sections were cut varying in thickness from 10 to 14 microns according to the requirements and stained in Heidenhain's iron-alum hæmatoxylin

Microsporangium

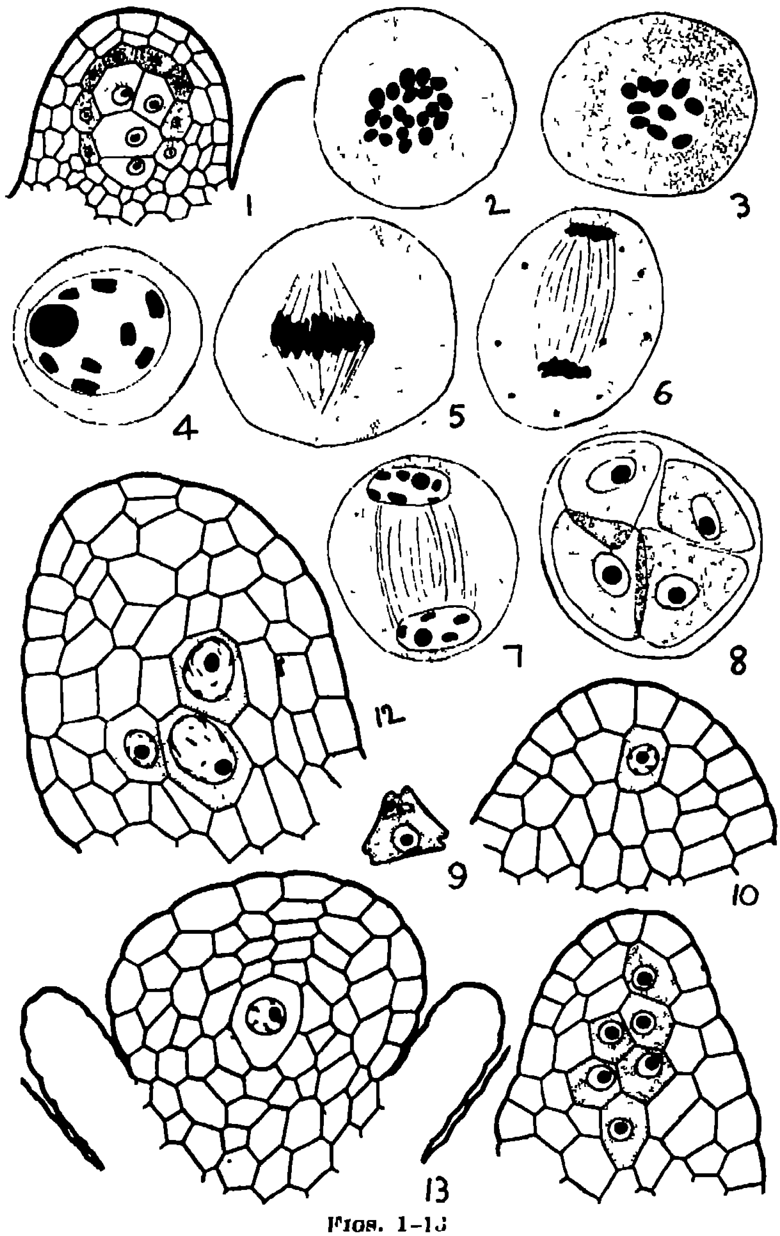
In the young anther the hypodermal layer becomes differentiated as the archesporium This divides into an outer primary parietal layer and an inner primary sporogenous layer The parietal layer by further divisions forms an endothecium, middle layers and a tapetum (Fig 1) In *Scutia myrtina* there are three middle layers whereas in *Z Jujuba* and *Z. Œnoplia* there are only two In both the species of *Zizyphus* the nuclei of the tapetal cells begin to divide when the microspore-mother cells begin to recover from synizesis as described by Cooper (1933) in certain Angiosperms Though they are usually binucleate, occasionally three to four nuclei are also seen. The primary sporogenous cells, by repeated divisions, form a number of microspore-mother cells

Microsporogenesis

The first sign of the entry of the nucleus into the meiotic prophase is shown by its increase in size accompanied by the formation of a system of chromatin threads No irregularities are seen when the meiotic divisons are taking place and they proceed normally (Figs 2–7) During diakinesis the univalents of a pair are attached at both ends (Fig 4) or sometimes at one end only. In *Z. Jujuba* (Fig 2) 20 and in *Z Œnoplia* (Fig 3) 10 bivalents were counted in metaphase plates In the second division the two spindles may be arranged either parallel to each other or at right angles, the latter condition being more common The tetrad of spores are separated by quadripartition furrows (Fig 8) which progress centripetally. At the time of shedding, each pollen grain has a large tube nucleus and a small slightly elongated generative nucleus (Fig. 9). The intine is thin and the exine thick and smooth The endothecial cells show fibrillar thickenings at the time of dehiscence

Female Gametophyte

Development of the female gametophyte was studied in detail in all the three plants under investigation. The nucellar primordia take their origin as lateral protuberances, grow horizontally for some distance, and then bend down finally becoming anatropous. By the time the integuments are differentiated a single hypodermal archesporial cell differentiates itself by



FIGS. 1-13

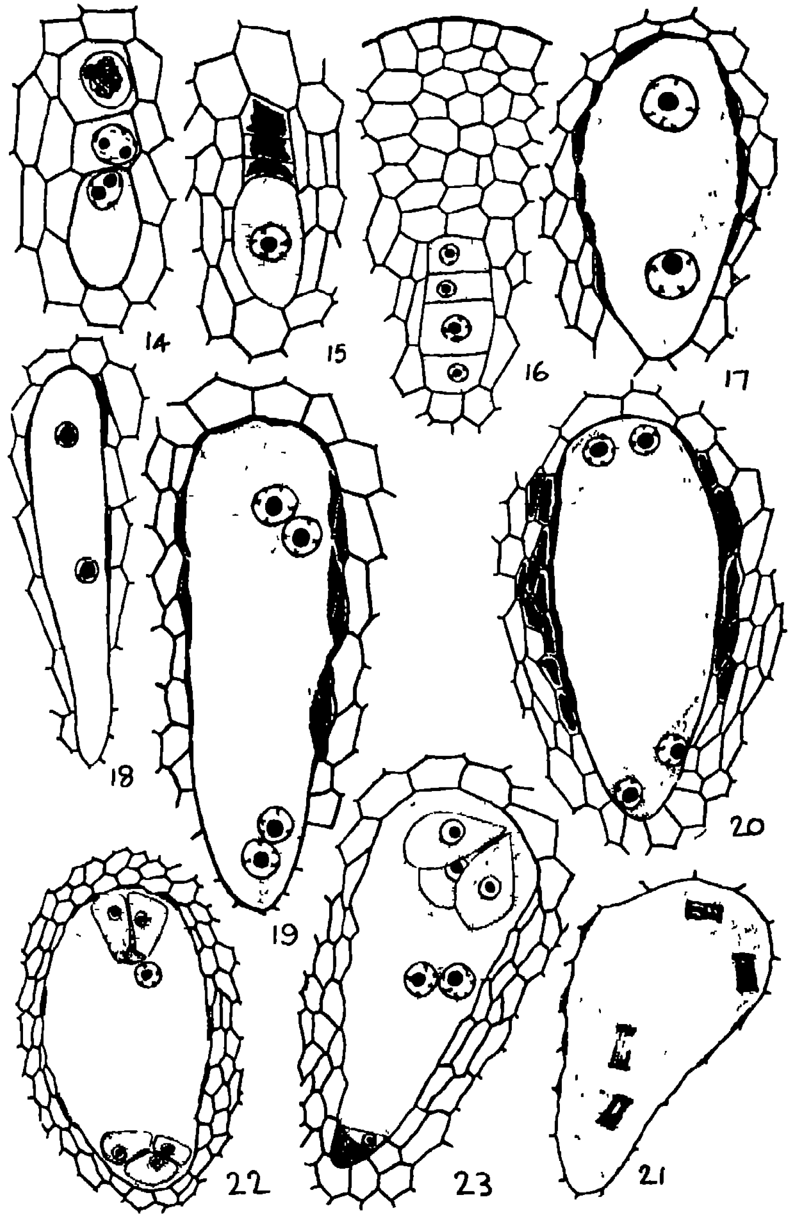
Fig. 1.—Transverse section of a young anther of *Z. Jujuba*. × 800. Figs. 2-8.—Stages in the development of the microspores of *Z. Jujuba* and *Z. Œnoplia*. × 2700. Fig. 9.—Pollen grain of *Z. Jujuba*. × 400. Fig. 10.—A single hypodermal archesporial cell of *S. myrtina*. × 1800. Fig. 11.—Multiple archesporium of *Z. Jujuba* × 1800. Fig. 12.—Three megaspore-mother cells of *Z. Jujuba*. × 1800. Fig. 13.—A megaspore-mother cell of *Z. Œnoplia*. × 1800.

its conspicuous size in *S myrtina* and in *Z Œnoplia* (Fig 10). In *Z. Jujuba* multiple archesporial cells are formed (Fig 11) as in *Z. sativa* (Chiarugi, 1930). In *Z Jujuba* there is a selection and the favourably situated archesporium develops further and becomes the megaspore-mother cell. It cuts off a number of parietal cells while the other archesporial cells degenerate gradually. Sometimes two or three archesporial cells are seen to develop up to the mother cell stage (Fig 12) In *S. myrtina* and *Z. Œnoplia* a single archesporial cell divides forming an outer primary parietal cell and an inner megaspore-mother cell. The former divides periclinally forming a number of parietal layers (Fig 13) The megaspore-mother cell by two successive divisions gives rise to a linear tetrad of megaspores In *Z Jujuba* and *S myrtina* invariably the chalazal megaspore is functional (Figs 14 and 15), while in *Z Œnoplia* the second or the third are also seen to develop further (Fig 16).

The functional megaspore increases in size soon becoming vacuolate and its nucleus by three successive divisions gives rise to a normal eight-nucleate embryo-sac (Figs. 17–24) The breaking down of the nucellar cells adjacent to the embryo-sac commences at the two-nucleate stage (Figs. 17 and 18) The synergids in *Z Œnoplia* and *Z Jujuba* are provided with a "filiform apparatus" in later stages (Fig 25), as in *Z sativa* (Chiarugi, 1930), but in *Scutia myrtina* it is absent In all the plants studied here, the egg is slightly larger than the synergids The antipodals are three in number with distinct cell walls and they are ephemeral degenerating soon after fertilization

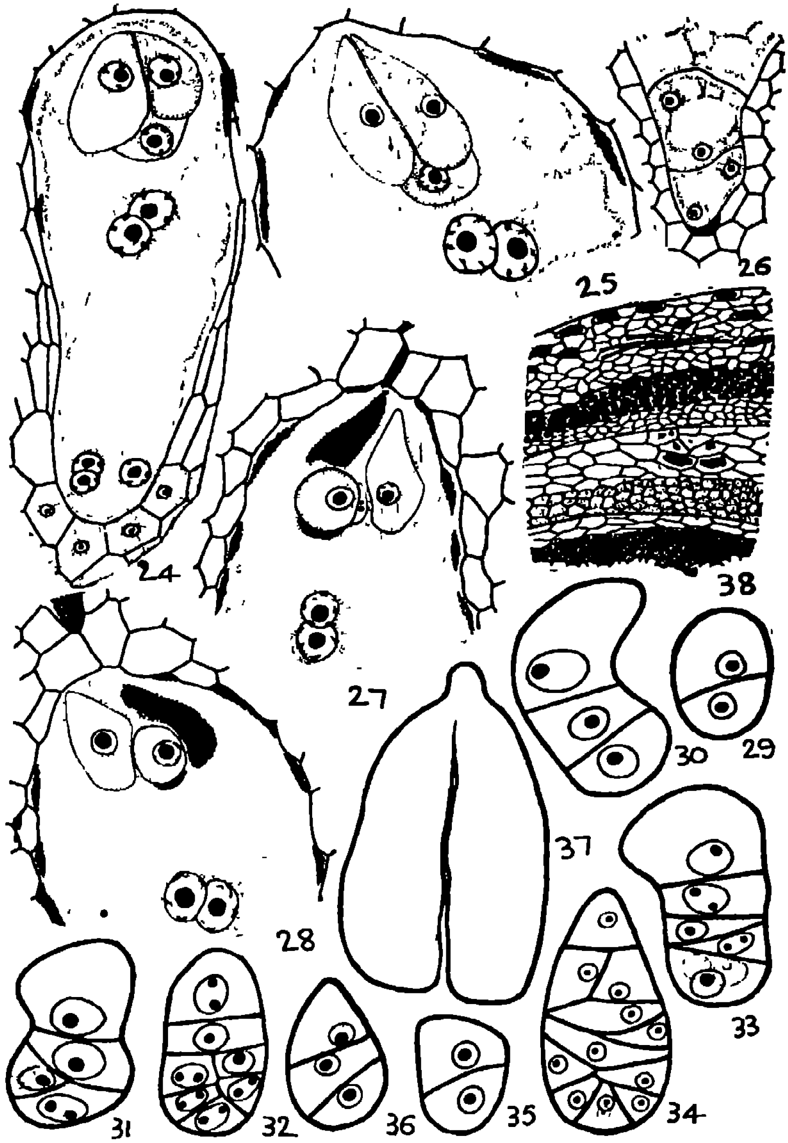
Variations are seen in the antipodals of *Z. Jujuba* They enlarge in size and the nuclei of one or two of them may divide, the resulting nuclei being separated by vacuoles (Fig 26) A similar phenomenon is reported in *Z sativa* (Chiarugi, 1930) According to Chiarugi one of the antipodals becomes four-nucleate in *Z sativa* In the two species of *Zizyphus* under study, only two-nucleate antipodals have been observed, and it is not unlikely that they may develop further into the four-nucleate stage

The massive nucellus is completely surrounded by the inner integument, while a small gap is left by the outer at the micropyle. The integuments and the nucellus are free from one another to the very base and the outer integument is supplied with vascular strands (Fig 38) The cells of the outer integument at the micropylar region divide actively so that it becomes thicker at this region whereas the inner integument remains practically of the same thickness throughout



FIGS. 14–23

Figs. 14, 15 and 16.—Tetrads of *Z. Jujuba*, *Scutia myrtina* and *Z. Œnoplia*. × 1800.
Figs. 17 and 18.—Binucleate embryo-sacs of *Z. Jujuba* and *Z. Œnoplia*. × 1800.
Figs. 19 and 20.—Four-nucleate embryo-sacs of *Z. Jujuba* and *Scutia myrtina*. × 1800.
Fig. 21.—All the nuclei dividing in the four-nucleate embryo-sac of *Z. Jujuba*. × 1800.
Figs. 22 and 23.—Fully developed embryo-sacs of *Z. Jujuba* and *Z. Œnoplia*. × 1800.



FIGS. 24–38

FIG. 24.—Eight-nucleate embryo-sac of *Scutia myrtina*. Note the dense cytoplasmic contents of the cells just beneath the embryo-sac × 1800. Fig. 25.—The egg apparatus and the polars of *Z. Jujuba*. Note the " Filiform apparatus " of the synergids. × 1800. Fig. 26.—Two, binucleate and one degenerated antipodals of *Z. Jujuba*. × 1800. Fig. 27.—The pollen tube has destroyed one of the synergids of *Z. Œnoplia*. × 1800. Fig. 28.—Pollen tube in the embryo-sac of *Z. Jujuba*. × 1800. Figs. 29–34.—Stages in the development of the embryo of *Z. Jujuba*. × 1800. Figs. 35 and 36.—Two- and three-celled embryos of *Z Œnoplia*. × 1800. Fig. 37.—Fully formed embryo of *Z. Jujuba*, showing the cotyledons and the radicle. × 20 Fig. 38.—Part of the mature seed showing oil-containing cells and vascular traces in the outer integument in *Z. Jujuba*. × 160.

Fertilization

Fertilization was observed only in *Z. Œnoplia* The male cell is long and its nucleus contains two nucleoli (Fig 27) Syngamy takes place as usual in the resting condition Stages of fertilization were not available in the other two plants but remnants of pollen tubes were seen in the embryo-sacs (Fig 28) In all the pollen tube has to pass through a large number of nucellar cells before reaching the embryo-sac

Endosperm Formation

The polars fuse either at the centre or at the micropylar end of the embryo-sac at the time of fertilization The endosperm formation is of the free nuclear type and the nuclei are distributed sparsely throughout the embryo-sac but slightly more in number towards the chalazal end They do not show any activity until the embryo begins to divide rapidly Wall formation commences at a very late stage when the embryo is a small undifferentiated mass of cells and its development is acropetal At the time the wall formation is initiated at the micropylar region, the rest of the endosperm nuclei become very active and divide very rapidly followed by wall formation Their activity seems to be very great at about two-thirds of the embryo-sac from the micropylar end The nuclei towards the chalazal end do not show any tendency to divide and remain free throughout This portion of the embryo-sac functions as a haustorial organ By the building of the endosperm tissue the long and narrow embryo-sac widens encroaching upon the adjacent nucellar cells When the cotyledons are differentiating in the embryo, the embryo-sac is several times wider than it was just before the commencement of wall formation in the endosperm

There is a remarkable growth of the embryo-sac after fertilization The organized eight-nucleate embryo-sac in *Z Jujuba* is about 60-70 microns in length, on an average. Soon after fertilization it begins to grow rapidly more towards the chalaza than towards the micropyle eating up the nucellar cells on its way. It also gets plenty of food both from the micropylar and the chalazal nucellar cells At a stage when the embryo is a spherical mass of cells the embryo-sac occupies the whole length of the ovule and measures about 3,000–3,500 microns, being thus roughly 50 times longer than what it was at the eight-nucleate stage A similar state of affairs is seen in *S myrtina* and *Z. Œnoplia*. In all the cases, the embryo-sac does not grow in width at this period. All the three plants show the hypostase at the chalazal region of the embryo-sac

Development of the Embryo

The fertilized egg undergoes a long period of rest When once the period of rest is broken, divisions follow rapidly. In *Z Jujuba* and *Z. Œnoplia*

the fertilized egg divides by a transverse wall forming two cells (Figs. 29 and 35). The distal cell divides once by a transverse wall forming a proembryo of three cells (Figs 30 and 36) Then a vertical division takes place in the first cell and in the resulting two cells intersecting oblique walls are laid (Figs 31, 32, 33 and 34) Further stages in the development of the embryo are difficult to trace By a series of divisions the embryo becomes a spherical mass of cells and later heart-shaped The basal cell divides forming a few suspensor cells In the ripe seed the embryo has plenty of reserve food in the perisperm The mature embryo has two long cotyledons and a short radicle (Fig 37) Though two ovules develop in a fruit, invariably one is very much smaller than the other Occasionally, only one is seen to develop well while the other degenerates

Perisperm

In the mature seed of *Z Jujuba* the nutritive tissue is not the endosperm but it is the perisperm The nucellar cells store plenty of nutrition and the embryo depends upon this reserve food for its further development, as in Piperaceæ, Nymphæaceæ, Polygonaceæ Aristolochiaceæ and such other families In *Z Jujuba* even the inner integuments show oil-containing cells

Discussion

The enormous increase in length of the embryo-sac towards the chalazal where there is a pad of tannin cells, and the deeply staining endosperm nuclei which remain free, show that the endosperm towards the chalazal end has a definite haustorial function It absorbs food materials from the surrounding nucellar cells and passes it on to the growing embryo

In all the plants studied here the integuments and the nucellus are free from one another to the very base, and the outer integument is supplied with vascular bundles Kershaw (1909) compared the vascular supply of the ovule of *Myrica Gale* with the outer series of vascular strands in the ovule of *Trigonocarpon* Some of the seeds of pteridosperms and all the fossil seeds have free nucelli and vascular integuments These are regarded as primitive characters Benson and Welsford (1909) working on *Juglans regia*, however, say " that there are several cases showing vascular supply similar to that described for *Myrica Gale* by Kershaw There is no adequate reason to doubt that a wider investigation would reveal numerous instances" The present investigation has brought to light one more instance in which the nucellus and the integuments are free and the outer integuments is supplied with vascular strands As the integuments are quite massive and as there is a great demand on them for food supply, the presence of vascular traces in

the outer integument is quite justifiable. In addition to these, in Rhamnaceæ there are several primitive characters such as the massive nucellus, plenty of tannin in the floral parts, and the mutiple archesporium

Summary

1 The hypodermal archesporium in the anther forms the sporogenous tissue after cutting off the parietal cells

2 The tapetal nuclei divide forming two to three nuclei in each tapetal cell

3 There are 20 bivalents in *Z Jujuba* and 10 in *Z Œnoplia*

4 Tetrad formation is effected by quadripartition furrows

5 Pollen grains are two-nucleate at the shedding stage

6. In the nucellus there is a single hypodermal archesporial cell in *Z Œnoplia* and *S myrtina* but in *Z Jujuba* multicellular archesporium is noticed In all the cases parietal cells are cut off by the archesporial cells

7 Embryo-sac is normal and the antipodals are organised into cells In *Z Jujuba* the antipodals occasionally enlarge followed by the division of their nuclei

8 The antipodal region of the embryo-sac penetrates deep into the nucellus and is nutritive in function

9 Intersecting oblique walls are formed in the embryo at a very early stage

10 In the mature seed there is perisperm

11 The nucellus and the integuments are free from one another

12 The outer integument has vascular supply

Acknowledgements

The writer is indebted to Professor M A Sampathkumaran, University of Mysore, for his guidance and encouragement and to Mr S B Kausik for his criticism and valuable assistance at several stages during the course of the investigation.

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THE MYXOPHYCEAE OF THE TRAVANCORE STATE, INDIA

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(*From the Department of Botany, Benares Hindu University*)

Received November 7, 1939
[Communicated by Professor Y Bhâradwâja, M.Sc., Ph.D. (London), F.L.S., F.N.I.]

There has been absolutely no record of algae from the Travancore State This communication deals with fifty-one forms of the Myxophyceae collected during the months of May and June, 1938, from the central and southern parts of the State, and five of them are new

Systematic Enumeration of the Species Observed

I Chroococcales

Chroococcaceae

Genus *Microcystis* Kützing

1 *Microcystis aeruginosa* Kütz Geitler in Rabenhorst's *Kryptogamen-flora von Europa*, XIV Band, Cyanophyceae, 1932, p 736, Fig 59 *d*

Diam cell., 3–5 μ.

Habitat.—Along with *Phormidium Jenkelianum*, in a stagnant pool, Vanchiyoor, Trivandrum

2. *Microcystis pulverea* (Wood) Forti Geitler, *op cit.*, 1932, p. 144, Fig. 64

Diam cell., 3·3 μ

Habitat.—On moist soil rich in lime, Perukada.

Genus *Aphanocapsa* Naegeli

3. *Aphanocapsa litoralis* Hansg Geitler, *op cit.*, 1932, p 152, Fig. 66 *b*

Diam. cell , 3·5–5 μ.

Habitat.—In brackish waters, Veli.

4. *Aphanocapsa rivularis* (Carmichael) Rabenhorst Tilden, *Minnesota Algae*, 1910, Vol. I, Pl II, Figs 8 and 9

Diam. cell., 5·5–7 μ.

The plant mass is blue green, cushion-like and attached to stones and water plants.

Habitat.—In a pool, Valiyathura, Trivandrum.

Genus *Aphanothece* Naegeli

5. *Aphanothece stagnina* (Spreng.) A. Braun Tilden, *op. cit.*, 1910, Pl. II, Fig. 15.

Lat. cell., 3·3–4·2 μ, long. cell., 6–7 μ.

Habitat —In a rice field, Attingal

Genus *Gloeothece* Naegeli

6. *Gloeothece rupestris* (Lyngbye) Bornet Tilden, *op. cit.*, 1910, Pl. II, Fig. 4.

Lat. cell., 4–4·5 μ, long cell., 6·6–9 μ.

Habitat.—On the sides of a flower-pot in the writer's house, Trivandrum.

Genus *Synechococcus* Naegeli

7 *Synechococcus elongatus* Naegeli. Geitler, *op. cit*, 1932, p. 273, Fig. 133 *a–c*.

Lat. cell, 2·5–3·3 μ; long cell., 3·3–6·6 μ.

Habitat —Attached to submerged rocks in a lake along with *Calothrix Elenkinii* and other algae, Vellayani.

II. HORMOGONALES

1 *Rivulariaceae*

Genus *Calothrix* Agardh

8. *Calothrix Elenkinii* Kossinskaja Geitler, *op. cit.*, 1932, p. 608, Fig. 383, 5 and 6

Lat fil., 12–18·5 μ; lat. trich, 6–8 5 μ; lat. cell, 6–8·5 μ; long. cell., 4–10 μ, lat het, 5 5-8 μ, long het, 6–9 μ

Habitat —Submerged and growing on a rock along with *Synechococcus elongatus* and other algae, Vellayani

2. *Scytonemataceae*

Genus *Plectonema* Thuret

9. *Plectonema Dangeardii* Frémy Frémy, *Les Myxophycées de l' Afrique équatoriale française*, 1930, p 177, Fig 148

Lat. fil., 1 6–2 μ; long cell., 2·5–3·3 μ, and rarely upto 9 μ long.

Habitat.—In stagnant water, Perukada

Genus *Tolypothrix* Kützing

10. *Tolypothrix lanata* Wartm. Geitler, *op cit*, 1932, p. 718, Fig. 459 *d*.

Lat. fil, 10–18 μ; lat. trich., 6·6–9·3 μ; long. cell., 4·5—19 μ; lat. het, 6–15 μ; long het., 8–33·8 μ.

Habitat.—In a pool, Veli.

Genus *Scytonema* Agardh

11. *Scytonema crispum* (Ag) Bornet Frémy, *op. cit*, 1930, p. 279, Fig. 257 *a*, *b* and *c*

Lat. fil., 16–21 μ ; lat trich., 10–12 μ ; long. cell., 3 3–6 μ, lat. het., 12–13·2 μ ; long. het., 6·6–13·5 μ

Habitat.—Attached to rocks in a brooklet, Nettayam

12. *Scytonema chiastum* Geitler Geitler, *op cit*, 1932, p. 751, Fig. 478

Forma. minor forma nov.

Lat. fil., 18–26·4 μ ; lat trich, 12–15 μ ; long cell., 3·3–6·6 μ, lat, het, 9–15 μ ; long. het, 15–17 μ.

Habitat.—In a rice field, Perukada

The alga differs from the type in smaller dimensions of all parts.

13 *Scytonema coactile* Mont Frémy, *op cit.*, 1930, p 301, Fig 258, *a*, *b* and *c*

Lat. fil, 19·8–23 μ ; lat trich, 12–15 μ, long cell, 6–8 μ ; lat het, 12–14 μ ; long het, 9–15 μ.

Habitat.—Attached to rocks near a spring, Nettayam

14. *Scytonema stuposum* (Kütz) Bornet Frémy, *op cit*, 1930, p 305, Fig. 260, *a* and *b*

Lat fil, 16–19·8 μ ; lat trich, 8–9 μ, long cell, 3·3–6 6 μ, lat het., 6·6–8 μ ; long het, 4·5–9·5 μ.

Habitat —On moist sandy soil, Tiruvala

15. *Scytonema ocellatum* Lyngbye Frémy, *op cit*, 1930, p 309, Fig. 263, *a* and *b*.

Lat fil., 15–19 μ ; lat trich, 9–14 μ, long cell, 3·3–6 6 μ ; lat het, 9–12 μ ; long. het., 12–19 μ.

Habitat.—On a brick wall, Trivandrum

3. *Oscillatoriaceae*

Genus *Spirulina* Turpin

16. *Spirulina princeps* W. and G. S West Geitler, *op cit.*, 1932, p. 928, Fig. 593 *d*.

Lat. trich., 3·3–4 5 μ, spot inter duo spir, 10·5–12 μ.

Habitat —Along with *Oscillatoria sancta* in a stagnant tank, Pallichal.

17. *Spirulina major* Kütz Frémy, *op. cit.*, 1930, p 235, Fig 208.

Lat. trich, 1·5–2 μ ; spot. inter. duo. spir., 2·5–6·6 μ.

Habitat.—Among other algae in a fresh-water pond, Veli.

Genus *Oscillatoria* Vaucher

18. *Oscillatoria nigro-viridis* Thwaites Tilden, *op. cit*, 1910, Pl. IV, Fig. 12.

Lat. fil, 3·3–7 μ; long. cell., 1·5–2·2 μ.

Habitat.—Along with other algae on the side of a pond, Veli

19. *Oscillatoria sancta* (Kütz) Gom Tilden, *op cit*, 1910, Pl IV, Fig. 5.

Forma tenuis forma nov.

Lat. trich, 6·6–7 μ; long. cell., 1·6–2 μ

Habitat—Along with *Spirulina princeps*, in a stagnant tank, Trivandrum.

The form differs from the type in smaller dimensions of the cells

20. *Oscillatoria obscura* Brühl et Biswas, *Journal of the Department of Science*, Calcutta, 1925, Vol. VII, Pl. II, Fig 13

Lat. fil, 3·3–4·5 μ, long cell., 1–1·6 μ

Habitat.—In a drain, along with other algae, Poojapura, and also in a water channel, Karamana

21 *Oscillatoria curviceps* Agardh Tilden, *op cit.*, 1910, Pl IV, Fig 7

Forma

Lat. fil., 6·6–11·1 μ, long cell, 1·5–2 μ

Habitat—In a rice field, Perukada

The form has slightly narrower trichomes and shorter cells

22. *Oscillatoria anguina* (Bory) Gom Tilden, *op cit*, 1910, Pl IV, Fig. 9.

Lat. fil, 5–6·6 μ; long cell, 1·6–2 μ

Habitat.—In a tank, Pallichal.

23. *Oscillatoria chalybea* Mertens Tilden, *op. cit.*, 1910, Pl. IV, Fig. 36.

Lat. fil., 6·6–13 μ; long cell., 3·3–4 μ

Habitat—In a tank, Kunnukuzi

Var *insularis* Gardner Geitler, *op. cit*, 1932, p 954, Fig 606 *c*.

Lat. trich, 6–7 μ; long cell, 1·6–4 μ

Habitat—In a drain, Trivandrum.

24. *Oscillatoria pseudogeminata* G Schmidle Geitler, *op. cit.*, 1932, p. 966, Fig 616

Lat fil., 1·6–2·4 μ; long cell., 1·6–3·3 μ

Habitat.—In a drain, Thampanoor, Trivandrum.

25. *Oscillatoria terebriformis* (Ag.) forma Rao. Rao, "The Myxophyceæ of the United Provinces, India—I," *Proc. Ind. Acad. Sci.*, 1936, Sec. B, Vol. 3, No. 2; (*cf.* Geitler, *op. cit.*, 1932, Fig. 607 *d*)

Lat. fil., 4–4·5 μ; long. cell., 3·3–4·5 μ

Habitat.—On water-logged soil, Palode.

26. *Oscillatoria Boryana* Bory. Geitler, *op. cit.*, 1932, p. 955, Fig. 607, *b* and *c*.

Lat. fil., 3·3–5 μ, long. cell., 4·9–6 μ.

Habitat.—Attached to rocks in the Karamana river, Vattiyoorkavu.

27. *Oscillatoria articulata* Gardner. Geitler, *op. cit.*, 1932, p. 963, Fig. 614.

Forma tenuis forma nov.

Lat. fil., 1·6–2·4 μ; long. cell., 1·3–1·6 μ

Habitat.—In a bath-room drain, Trivandrum.

The form differs from the type in having narrower trichomes and shorter cells.

28. *Oscillatoria Geitleri.* Frémy, *op. cit.*, p. 221, Fig. 185.

Lat. fil., 3·5–4·5 μ, long. cell., 3·3–4 μ

Habitat.—In paddy fields, Palode.

29. *Oscillatoria okeni* Agardh. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 35.

Lat. fil., 6–7·5 μ, long. cell., 3–4·6 μ.

Habitat.—In a roadside drain, Trivandrum.

30. *Oscillatoria formosa* Bory. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 33.

Lat. fil., 3·7–4·9 μ, long. cell., 3·3–5 μ.

Habitat.—On the sides of a canal, Pangode.

31. *Oscillatoria subproboscidea* W. and G. S. West. Geitler, *op. cit.*, 1932, p. 969, Fig. 618 *c*.

Lat. fil., 4·2–6 μ, long. cell., 3·3–4 μ

Habitat.—Attached to a rock in a watercourse, Nettayam.

Genus *Phormidium* Kützing

32. *Phormidium foveolarum* (Montagne) Gom. Geitler, *op. cit.*, 1910, Pl. IV, Fig. 51.

Lat. fil., 1·5–1·7 μ; long. cell., 0·8–2 μ

Habitat.—On rocks run over by a shallow stream, Nettayam.

33 *Phormidium Jenkelianum* G. Schmidle Geitler, *op. cit.*, 1932, p. 1001, Fig 638

Lat fil, 4–6 6 μ, long cell, 3·3–4 μ.

Habitat —Along with *Microcystis aeruginosa*, in the bath-room drain of the writer's house, Trivandrum

34 *Phormidium Retzii* (Ag.) Gom Tilden, *op. cit.*, 1910, Pl. V, Figs 1–4.

Lat. trich., 4–6·6 μ, long. cell., 4–9·9 μ.

Habitat —On cocoanut logs submerged in a water channel, Pallichal; also on soil at the foot of hills, Perukada and Nedumangad.

Forma major forma nov

Lat fil, 6·6–10 5 μ; long cell, 3·6–9·9 μ.

Habitat —Growing on soil, Trivandrum and environs

The form differs from the type in bigger dimensions of the filaments and cells.

35 *Phormidium calcicola* Gardner Geitler, *op cit*, 1932, p 1012, Fig 646 *a*.

Lat fil, 4·9–6 μ, long cell., 6–7 μ

Habitat.—On rocks in a watercourse, Nettayam

36 *Phormidium ambiguum* Gom Tilden, *op cit*, 1910, Pl. V, Fig. 5

Lat fil, 4·2–6·6 μ; long cell, 3–3·3 μ

Habitat —On rocks, submerged in water from a spring, Nettayam

37. *Phormidium corium* Gom Tilden, *op. cit*, 1910, Pl. IV, Figs. 71 and 72

Lat. fil, 3·5–6 6 μ, long cell, 4–7 μ

Habitat.—In rice fields, Palode.

38. *Phormidium inundatum* Kütz Tilden, *op. cit.*, 1910, Pl. IV, Figs 69 and 70.

Lat fil, 3·3–4 μ; long. cell., 3–3·3 μ

Habitat.—From a gutter, Trivandrum

39. *Phormidium fragile* (Menegh.) Gom Tilden, *op. cit.*, 1910, Pl. IV, Figs 52 and 53.

Lat. fil., 1·5–1·7 μ; long. cell., 1·6–1·9 μ.

Habitat.—In a roadside drain, Poojapura, Trivandrum.

Genus *Lyngbya* Agardh

40. *Lyngbya Lachneri* (Zimmermann) Geit Geitler, *op. cit.*, 1932, p. 1037, Fig. 655.

Lat. fil., 4·2–6·6 μ, long. cell., 1·6–2·5 μ.

Habitat.—Attached to rocks in a brooklet, Nettayam

41. *Lyngbya polysiphoniae* Frémy Frémy, *op. cit*, 1930, p 184, Fig. 153.

Lat fil., 3–3·3 μ; long cell, ·8–1 μ.

Habitat.—In a tank, Peroor

42. *Lyngbya Borgerti* Lemm Geitler, *op. cit*, 1932, p 1047, Fig. 662 *a*.

Lat. fil, 4·5–6 μ, long. trich, 4 1–4·9 μ, long cell, 1–1·6 μ.

Habitat—In a paddy field, Attingal

43. *Lyngbya Schakletoni* W and G S. West. Geitler, *op cit*, 1932, p. 1047, Fig. 662 *c*.

Lat. fil., 13–15 μ, lat. trich., 9–10 μ; long cell, 1·6–3 μ.

Habitat.—In a pool, Veli.

44. *Lyngbya Hieronymusilemm.* Lemmermann, *Kryptogamenflora der Mark Brandenburg*, 1910, p. 102, Fig 6

Forma robusta forma nov

Lat. fil, 26·4–33 μ, lat trich, 15-17 μ. long cell, 3–3·5 μ

Habitat.—In a stagnant pool, Chabara

The alga differs from the type in having much broader trichomes, wider sheaths and slightly longer cells.

45. *Lyngbya Birgei* G. M Smith Geitler, *op cit*, 1932, p 1048, Fig. 663

Lat. fil, 23·1–26 4 μ, lat. trich, 22–24 μ, long cell, 3–3 9 μ.

Habitat.—In paddy fields, Perukada, and also on wet mud of a drying tank, Allepy

46. *Lyngbya ochracea* Thur. Geitler, *op cit*, 1932, p 1045, Fig. 661 *d*

Lat. fil, 3·3–4 μ, lat. trich, 0·8–1 μ, long cell., 0·4–0·8 μ.

Habitat.—On rocks submerged in water at the bottom of a hill, Aruvikkara.

47. *Lyngbya ceylanica* var. *constricta* Frémy Frémy, *op cit.*, 1930, p. 185, Fig. 151.

Lat. fil., 27–30 μ; lat trich., 19-23 μ; long cell., 6·6–10 μ.

Habitat.—In a stagnant pool, Veli.

48. *Lyngbya semiplena* (Ag.). Geitler, *op. cit*, 1932, p. 1060, Fig. 672 *a*.

Lat. fil., 12–17 μ; lat. trich., 6·6–10·8 μ; long cell, 2·5–3·9 μ

Habitat —In a stagnant pond, Veli.

49. *Lyngbya aerugineo-coerulea* (Kütz.) Gom. Frémy, *op. cit.*, 1930, p. 193, Fig 157

Lat. fil, 6·3–6·6 μ; lat. trich, 4·6–5 μ; long. cell., 3·3–4·2 μ.

Habitat —On rocks near a water fall, Aruvikkara.

The alga possesses slightly shorter cells than those of the type

50. *Lyngbya Martensiana* Menegh. Frémy, *op. cit*, 1930, p. 193, Fig. 158.

Lat fil, 9·5–10 μ, lat. trich, 9–9·5 μ; long. cell., 3·5–5 μ.

Habitat —In a stagnant tank, Perukada.

Var. *minor* Gardner Geitler, *op. cit*, 1932, p. 1063, Fig 675 *c*.

Lat fil, 6 6–8 μ; lat. trich, 6–7·5 μ; long. cell, 0·8–1 μ

Habitat —In a pool, Allepy

51 *Lyngbya magnifica* Gardner Geitler, *op cit.*, 1932, p 1067, Fig. 680 *b*.

Lat. fil, 25–36 μ; lat. trich, 20–33 μ; long. cell, 3·5–6·6 μ

Habitat.—Along with *Aphanocapsa litoralis* in a tank, Veli.

In conclusion, the writer wishes to express her great indebtedness to Professor Y. Bharadwaja for his kind guidance and criticism throughout the course of this investigation.

THE MYXOPHYCEAE OF THE DELHI PROVINCE, INDIA—I

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IN the month of November 1938 the writer made a collection of Algae from Delhi and its suburbs. In this paper thirty-eight forms of the Myxophyceae have been described, of which five forms are new

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED

I CHROOCOCCALES

Chroococcaceae

Genus *Microcystis* Kützing

1. *Microcystis flos-aquae* (Wittr.) Kirchn Geitler in Rabenhorst's *Kryptogamen-flora von Europa*, Band XIV, Cyanophyceae, 1930–32, p 136, Fig 59 *e*, Crow, " The taxonomy and variation in the genus *Microcystis* in Ceylon," *New Phytologist*, 1923, Vol. 22, No 2, Pl. I, *b* & *c*, Tilden, *Minnesota Algae*, 1910, Vol. I, Pl II, Fig. 18

Lat. cell., 3·3–6 μ

Habitat.—In a pond, Delhi.

2 *Microcystis aeruginosa* Kütz Geitler, *op cit*, 1930–32, p. 136, Fig. 59 *d*; Crow, *op cit.*, 1923, Pl I, Fig. *a*, Tilden, *op. cit.*, 1910, Pl. II, Figs 21 and 22.

Lat. cell., 3·3-5·2 μ

Habitat —Free-floating in a pond, Delhi

3. *Microcystis scripta* (Richter) Geitler Geitler, *op cit*, 1930–32, p. 139, Fig. 62 *a*.

Forma.

Lat. cell., 3·3–6·6 μ

Habitat.—Free-floating in a pond, Delhi.

The form differs from the type in having smaller cells and in being more perforated.

Genus *Chroococcus* Naegeli

4. *Chroococcus montanus* Hansg. *forma* Rao C. Bhashyakarla Rao, " The Myxophyceae of the United Provinces, India—III, " *Proc. Ind. Acad. Sci*, Vol. VI, Sec. B, No. 6, 1937, p. 345, Fig 1 *F*.

Lat cell., 3·3–6 6 μ ; long. cell, 4–8 μ ; lat cell, cum. vag, 6–10 μ ; long cell cum. vag, 6–12 μ ; lat col, upto 16·5 μ

Habitat.—Along with *Microcoleus lacustris forma*, on a cemented floor near a tap, New Delhi

Genus *Gloeocapsa* Kützing

5. *Gloeocapsa stegophila* (Itzigs) Rabenh Geitler, *op. cit*, 1930–32, p 197, Fig 91 *b* ; Tilden, *op cit*, 1910, Pl I, Fig 24.

Forma

Lat col., 9–19 μ (–22 μ); lat cell., 3–5 5 μ ; cross-sheath, 1·5-2 μ

Habitat.—Free-floating in a pond along with *Oscillatoria pseudogeminata* and *O. chalybea*, Delhi

In this form the outline of the sheath is ragged.

Genus *Synechocystis* Sauvageau

6. *Synechocystis aquatilis* Sauvageau Tilden, *op cit.*, 1910, Pl I, Fig 10.

Lat. cell, 4–6·5 μ.

Habitat.—Free-floating along with *Pediastrum* species and sterile *Anabaena* in one of the tanks opposite to Secretariat Buildings, New Delhi.

The form is rather rarely met with in this collection

Genus *Merismopedia* Meyen.

7. *Merismopedia tenuissima* Lemm Geitler, *op cit*, 1930–32, p. 263, Fig. 129 *b*.

Lat. col, upto 16·5 μ, long col., upto 29·7 μ, lat cell., 2·5 μ.

Habitat —Planktonic in one of the fountains opposite Viceregal Lodge, New Delhi

8 *Merismopedia punctata* Meyen Geitler, *op cit*, 1930–32, p 264, Fig. 129 *c*

Lat col., upto 82·5 μ, long col, upto 115 5 μ, lat. cell, 3·3–3·5 μ ; long. cell., 3·3 μ.

Habitat —Free-floating along with *Oscillatoria princeps* and *O. sancta*, Delhi.

II. CHAMAESIPHONALES

Dermocarpaceae

Genus *Dermocarpa* Crouan

9 *Dermocarpa sphaerica* S et G Geitler, *op. cit*, 1930–32, p. 393, Fig 217.

Forma

Lat cell., upto 16·5 μ (–23·1 μ).

Habitat—Epiphytic on *Pithophora* species growing in a pond by the side of the Delhi Railway Station.

The form has slightly bigger sporangia with a thick wall

III HORMOGONALES

(*a*) *Scytonemataceae*

Genus *Tolypothrix* Kutzing

10. *Tolypothrix tenuis* Kütz *forma* Bhâradwâja Bhâradwâja, "The taxonomy of *Scytonema* and *Tolypothrix*, including some new records and new species from India and Ceylon," *Revue Algologique*, 1934, p 176; Geitler, *op. cit*, 1930–32, p 116, Fig 453 *a*

Lat fil, 6–9 μ; lat. trich, 4–6·8 μ, lat. het, 5·7·3 μ, long het., 6–18 μ; long. cell., 3–6·6 μ; (–10 μ)

Habitat—Free-floating in a pond along with *Aulosira* species, Delhi

Genus *Scytonema* Agardh

11 *Scytonema Pascheri* Bhâradwâja Bhâradwâja, *op cit*, 1934, p 163, Fig *c* and *d*

Lat fil., 13 2–16 5 μ, lat trich, 8–10 μ, (—13 2 μ); lat het, 8–10 μ, long. het, 9·9–16·8 μ, crass vag, 1·8–2 μ, when old upto 6 μ

Habitat—On moist soil along with sterile *Anabaena*, New Delhi

The form has slightly narrow filaments and smaller heterocysts

(*b*) *Nostocaceae*

Genus *Anabaena* Bory

12. *Anabaena Iyengari* Bhâradwâja var *tenuis* Rao C Bhashyakarla Rao, *op cit.*, 1937, p. 361, Fig 5 *a*, *b* & *c*

Lat. trich, 3·6–4 μ, long cell., 2·5–4 5 μ, lat het, 4 6–8 μ; long het., 4·8–12 μ; lat. spor., 10–22 μ (–24 μ).

Habitat.—Free-floating in a tank, along with *Anabaena* species, Delhi.

13 *Anabaena sphaerica* Born. et Flah. var. *attenuata* Bhâradwâja Bhâradwâja, "The Myxophyceae of the United Provinces, India—I," *Proc. Ind. Acad Sci.*, Vol. II, Sec. B, No. 1, 1935, p. 104, Fig 5 *G* and *H*.

Lat. cell, 4–4·5 μ; long. cell., 3–4·5 μ; lat. het., 5 5–6 μ; lat. spor., 9·9–10 μ; long spor, 10–13·2 μ

Habitat.—In a fountain opposite the Secretariat, New Delhi.

(*c*) *Oscillatoriaceae*

Genus *Spirulina* Turpin

14 *Spirulina maior* Kütz. Geitler, *op cit*, 1930–32, p. 930, Fig. 595; Frémy, "Les Myxophycees de Madagascar," *Annales de Cryptogamie exotique*, t. iii, Fasc IV, 1930, p 235, Fig 208; Carter, "A comparative study of the algal flora of two salt marshes, Part II," *Journal of Ecology*, 1933, Vol. XXI, p. 159, Fig. 2.

Lat trich, 1–1·5 μ; lat spir, 2·5–4 μ, spat inter duo. spir., 2·7–3·8 μ.

Habitat—On a moist soil along with other species of *Spirulina*, *Oscil.*, *latoria Martinii*, *O princeps* and *O pseudogeminata*, on the way to Qutab-Minar, Delhi.

15. *Spirulina subsalsa* Oerst Geitler, *op cit.*, 1930–32, p. 928, Fig 593 *a*.

Lat. trich, 1·5-2·3 μ; lat spir, 3–4 μ

Habitat—Along with *O. pseudogeminata* and others, on the way to Qutab-Minar, Delhi

Genus *Oscillatoria* Vaucher

16 *Oscillatoria proboscidea* Gom Tilden, *op. cit*, 1910, Pl IV, Fig. 4.

Lat trich, 14–16 μ; long cell, 2·5-3 μ

Habitat.—In a small collection of water along with *O. formosa*, Ridge, New Delhi.

17. *Oscillatoria chalybea* Mertens Geitler, *op cit*, 1930–32, p. 956, Fig 606 *c*

Lat. trich., 7·2–8 5 μ; long cell, 2·5–4 μ.

Habitat—Along with *O pseudogeminata* and *O amphigranulata* on moist soil near a well, Delhi.

18. *Oscillatoria pseudogeminata* G. Schmid. Geitler, *op cit*, 1930–32, p. 966, Fig. 616.

Forma.

Lat. trich., 1·9–2·2 μ; long. cell, upto 3·5 μ

Habitat.—On moist soil along with *O chalybea*, Delhi

The form has single granule on either side of the cross wall

19 *Oscillatoria formosa* Bory Tilden, *op. cit*, 1910, Pl. IV, Fig 33

Lat. trich, 4–6 μ; long. cell., 2–4 μ.

Habitat.—In a small water collection along with *O proboscidea*, Ridge, New Delhi.

20. *Oscillatoria anguina* (Bory) Gom Tilden, *op cit*, 1910, Pl. IV, Fig. 9.

Lat. trich., 4·5–6·8 μ; long cell., 1 6–3·3 μ.

Habitat.—On moist soil near a well on the way to Hauz-i-Khas, Delhi

21. *Oscillatoria Martinii* Frémy Frémy, *op cit*, 1930, p. 229, Fig. 203

Lat. trich., 5·5–6 μ; long cell, 1·6–1·8 μ

Habitat.—On moist edges of a pond along with *Lyngbya Martensiana* var. *calcarea* and *O terebriformis*, Roshenara Gardens, Delhi

22. *Oscillatoria brevis* (Kütz) Gom. Tilden, *op. cit.*, 1910, p. 79, Pl. IV, Fig. 32; Frémy, *op cit.*, 1930, p 225, Fig. 195

Lat. cell., 4–6 μ; long cell, 2–3 μ

Habitat.—In a small collection of water, Ridge, New Delhi

23. *Oscillatoria princeps* Vauch Geitler, *op. cit*, 1930–32, p 947, Fig 598 *a*

Lat. cell., 36·3–42·9 μ; long cell., 3–6·6 μ

Habitat.—Along with *Merismopedia punctata*, *Oscillatoria sancta*, and *Pediastrum* species, New Delhi.

24 *Oscillatoria sancta* (Kütz) Tilden, *op cit.*, 1910, Pl IV, Fig. 5

Lat. cell., 13–14 μ; long cell, 2–4·5 μ.

Habitat—Along with *O. princeps* and *Merismopedia punctata* and *Pediastrum* species, New Delhi.

25. *Oscillatoria cortiana* Mengh. Tilden, *op. cit*, 1910, Pl. IV, Fig. 34.

Lat cell., 3–5·3·8 μ; long cell., 2–2·8 μ.

Habitat.—On the edges of a pond, Roshenara Gardens, Delhi.

26 *Oscillatoria terebriformis* Ag Geitler, *op. cit*, 1930–32, p. 955, Fig. 607 *d*.

Lat cell, 4·5–5·5 μ; long. cell, 2·5–3·6 μ

Habitat.—On the Ridge, New Delhi

27. *Oscillatoria Okeni* Ag Geitler, *op cit*, 1930–32, p 956, Fig. 608 *a*.

Lat trich., 6·5–9 μ; long. cell, 2·8–3·6 μ (–6 μ)

Habitat —In a pond by the side of the Railway Station, Delhi.

28 *Oscillatoria Boryana* Bory Geitler, *op. cit*, 1930–32, p. 955, Fig. 607 *b* & *c*

Lat. trich, 4–6·6 μ, long. cell, 2–4 μ

Habitat —Near a well, on moist soil, on the way to Qutab-Minar, Delhi.

29. *Oscillatoria variabilis* Rao C. Bhashyakarla Rao, "The Myxophyceae of the United Provinces, India—II," *Proc. Ind Acad. Sci*, Vol. III, Sec. B, No 2, 1936, p. 172, Fig 3, *A–D*.

Lat trich, 5·4–6 μ; long cell, 2·3–3·5 μ.

Habitat —On moist soil, along with other species of *Oscillatoria*, Delhi.

30. *Oscillatoria tenuis* Ag Tilden, *op cit*, 1910, Pl IV, Fig 17

Lat trich, 5–6·6 μ, long. cell., 2·5–3 μ

Habitat —In a pond near Railway Station, Delhi

Genus *Phormidium* Kützing

31. *Phormidium tenue* (Menegh) Gom Geitler, *op cit*, 1930–32, p. 1004, Fig. 624 *d*

Lat. fil, 1 5–1 9 μ, long cell, 3–3·6 μ

Habitat —On moist soil, Delhi

32 *Phormidium fragile* Gom Tilden, *op cit.*, 1910, Pl IV, Figs. 52 and 53

Lat cell, 1·5 μ; long cell, 1–1·7 μ

Habitat.—On moist soil along with *Lyngbya Allorgei*, Delhi.

33. *Phormidium foveolarum* Gom Tilden, *op cit*, 1910, Pl IV, Fig. 54.

Lat. trich, 1·8–2 μ, long. cell, 2–2·5 μ.

Habitat.—On moist soil near a well on the way to Qutab-Minar, Delhi.

Genus *Lyngbya* Agardh

34. *Lyngbya Lagerheimii* (Möbius) Gom. Tilden, *op cit*, 1910, Pl. V, Fig. 23.

Lat. fil., 2·7 μ ; long. cell, 2–3·2 μ.

Habitat.—Free-floating in a tank, Delhi.

35 *Lyngbya majuscula* Harv. Geitler, *op. cit*, 1930–32, p. 1060, Fig. 672 *c, d*.

Lat. fil., 23·1–25·5 μ ; lat. trich., 9 9–13·2 μ ; crass. vag., 1·5–2·5 μ.

Habitat.—Free-floating in a pond along with *Pithophora* species, *Nitella* species and *Gloeocapsa stegophila*, Delhi

36. *Lyngbya Martensiana* Menegh. var *calcarea* Tilden Tilden, *op. cit.* 1910, Pl. V, Fig 44.

Lat. fil. 7·2–8 μ ; long. cell., 4–8 μ.

Habitat.—Free-floating in a pond along with *Oscillatoria Martinii*, and *O. terebriformis*, Delhi.

37. *Lyngbya lutea* (Ag) Gom Frémy, "Les Cyanophycces des Cotes d'Europe," *Memoires de la Societe Nationale des Sciences Naturelles et Mathematiques de Cherbourg*, 1934, tome XLI, Pl 28, Fig 4 *a* and *c*, Geitler, *op cit.*, 1930–32, p. 1058, Fig 670 *a* and *b*

Lat. fil, 5·5–7·6 μ ; lat trich, 4–4 5 μ ; long. cell., 3 3·5 μ.

Habitat —Free-floating in a pond, Delhi

Genus *Microcoleus* Desmaziers

38. *Microcoleus lacustris* (Rabenh) Farlow Geitler, *op. cit*, 1930–32, p. 1142, Fig 749

Forma.

Diam. cil., 30–45 μ ; lat trich., 2–3 μ ; long. cell, upto 6 μ.

Habitat.—On the cemented floor near a pipe along with *Chroococcus montanus* and *Calothrix* species, Delhi.

The form has smaller cells and more acutely tapering apical cell

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bhâradwâja for his kind guidance and criticism throughout the course of this investigation.

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STUDIES IN THE ANALYSIS OF FERTILISER EFFECTS

II. Photosynthetic Efficiency of *Saccharum officinarum* Leaves as Influenced by Certain Manurial Treatments

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Introduction

WHILE investigating the influence of different manurial treatments upon the growth and yield of plants it was felt necessary to have a complete physiological analysis of the fertilizer effect with respect to different economic crop plants The investigations conducted in this direction have given ample evidence to the fact that the fertilisers influence the different physiological activities of plants, especially transpiration and water requirement,[6,8,11,14] respiration and photosynthesis,[1,2,7,9,12] carbohydrate and nitrogen metabolism in general [3,4,5]

Direct evidences upon the photosynthetic activity of *Andropogon sorghum* as influenced by fertilizer treatments have been discussed in detail elsewhere [15] In the following pages, however, the observations recorded on *Saccharum officinarum* grown under different treatments with inorganic fertilizer and organic bulky manure are presented

The experiments were conducted under three distinct conditions, the medium of growth in all cases being the farm soil (sandy loam) In the first series the plants were grown in cemented pots 18″ × 12″ in size, and supplied with different inorganic fertilizers In the second series dealing with organic bulky manures the sugarcane plants were grown in concrete tanks 6′ × 6′ × 6′ in size. In the third series, the treatments were all given under field conditions Twenty-seven different combinations of nitrogen, phosphoric acid and potash with three levels of manuring in each case were confounded together. The lay-out in this series will be discussed in detail in a separate communication The cane under each treatment covered 1/45 acre area.

Towards the stage of maturity, when the plants attained an age of 260 days, the third leaf from the top of the main (primary) shoot from each

of the treatments under consideration, was collected. The rate of assimilation of the leaves thus collected was studied under optimal conditions* of CO_2 (0·25–0·30%), temperature (31° C. ± 0·2) and light (1500 C.P. ½ W. Phillips bulb at 18 cm. distance). Continuous current method with Blackman's commutator was used for studies on assimilation and respiration. Chlorophyll content was estimated after Oltman.[10]

Sampling methods for the study of photosynthetic efficiency of leaves were tried separately.[17] It was found that in cases where the effect of age was not the primary consideration, the collection of third leaf from the top of the main shoot towards the period of maturity and the measurement of photosynthesis under optimal conditions in such leaves gathered from different treatments gave a more valid comparison of the effects of two or more treatments, as compared with at random selection of leaves from plants grown under varied fertilizer treatments. In view of this as also in view of the fact that a detailed study of age factor has already been reported earlier[16] no attempt was made to work out once again the effect of age on the assimilation rate of cane leaves Three separate estimations were made of the photosynthetic efficiency of the leaves, and the significance of the values thus obtained, tested by the method of analysis of variance. The interaction effects of nitrogen, phosphoric acid, and potash and on assimilating efficiency, shall be dealt with in a subsequent contribution alongside the effect of similar treatments on growth and yield of plants.

In all these different series proper care was taken to grow the plants under as uniform condition of soil moisture as practicable, and to perform such other intercultural operations as hoeing, weeding, earthing, etc., whenever necessity was felt.

Details of Treatments

No. I. Pot Series—

(i) Control . supplied with no fertilizer.

(ii) N_2-fed plants : supplied with 6·0 gms. of ammonium sulphate per pot

(iii) P_2O_5-fed plants : supplied with 2·0 gms of double superphosphate per pot.

(iv) K_2O-fed plants : supplied with 2·0 gms of potassium sulphate per pot.

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* The optimal values for these factors were determined earlier in connection wit another series of investigations after the manner discussed in another paper.[18]

(v) N–K-fed plants : supplied with 6·0 gms of ammonium sulphate and 2·0 gms. of potassium sulphate per pot

(vi) N–P-fed plants supplied with 6·0 gms. of ammonium sulphate and 2·0 gms of double superphosphate per pot.

(vii) P–K-fed plants : supplied with 2 0 gms of double superphosphate and 2·0 gms. of potassium sulphate per pot

(viii) N–P–K-fed plants . supplied with 6 0 gms. of ammonium sulphate, 2·0 gms of double superphosphate and 2·0 gms of potassium sulphate per pot

No. II. Concrete-tank Series—

(i) Control . no organic bulky manure was supplied
(ii) Night-soil-fed plants.
(iii) Castor cake-fed plants
(iv) Sheep-dung-fed plants
(v) Cow-dung-fed plants
(vi) Compost-fed plants

In all these cases the quantity of different manures was calculated on nitrogen basis and supplied at the rate of 150 lbs nitrogen per acre.

No. III Field Series—

(i) Control · plots supplied with no artificial fertilizer

Single fertilizer-fed plants

(ii) N_2-fed plants : supplied with—

(*a*) 75·0 lbs. of nitrogen per acre . ..	(N_1)
(*b*) 150·0 lbs of nitrogen per acre .. .	(N_2)

(iii) P_2O_5-fed plants · supplied with—

(*a*) 40·0 lbs. of P_2O_5 per acre . ..	(P_1)
(*b*) 80·0 lbs of P_2O_5 per acre .	(P_2)

(iv) K_2O-fed plants : supplied with—

(*a*) 40·0 lbs of K_2O per acre	(K_1)
(*b*) 80·0 lbs of K_2O per acre . ..	(K_2)

Double fertilizer-fed plants

(v) N–P-fed plants : supplied with—

(*a*) 75·0 lbs. of N_2 and 40·0 lbs of P_2O_5 per acre	(N_1P_1)
(*b*) 75·0 lbs of N_2 and 80·0 lbs of P_2O_5 per acre	(N_1P_2)
(*c*) 150·0 lbs. of N_2 and 40·0 lbs. of P_2O_5 per acre	(N_2P_1)
(*d*) 150·0 lbs of N_2 and 80·0 lbs. of P_2O_5 per acre	(N_2P_2)

(vi) N–K-fed plants : supplied with—

(*a*) 75·0 lbs. of N_2 and 40·0 lbs. of K_2O per acre (N_1K_1)
(*b*) 75·0 lbs of N_2 and 80·0 lbs of K_2O per acre (N_1K_2)
(*c*) 150·0 lbs of N_2 and 40·0 lbs of K_2O per acre (N_2K_1)
(*d*) 150·0 lbs of N_2 and 80·0 lbs of K_2O per acre (N_2K_2)

(vii) P–K-fed plants : supplied with—

(*a*) 40·0 lbs of P_2O_5 and 40·0 lbs of K_2O per acre (P_1K_1)
(*b*) 40·0 lbs of P_2O_5 and 80·0 lbs. of K_2O per acre (P_1K_2)
(*c*) 80 0 lbs of P_2O_5 and 40·0 lbs of K_2O per acre (P_2K_1)
(*d*) 80·0 lbs. of P_2O_5 and 80 0 lbs. of K_2O per acre (P_2K_2)

Three fertilizer-fed plants.

(viii) N–P–K-fed plants : supplied with—

(*a*) 75·0 lbs of N_2, 40·0 lbs. of P_2O_5 and 40·0 lbs of K_2O per acre .. .. .. ($N_1P_1K_1$)
(*b*) 150·0 lbs. of N_2, 40·0 lbs of P_2O_5 and 40·0 lbs. of K_2O per acre .. . .. ($N_2P_1K_1$)
(*c*) 75·0 lbs of N_2, 80 0 lbs. of P_2O_5 and 40·0 lbs of K_2O per acre .. .. .. ($N_1P_2K_1$)
(*d*) 75·0 lbs. of N_2, 80·0 lbs of P_2O_5 and 80·0 lbs of K_2O per acre .. .. .. ($N_1P_2K_2$)
(*e*) 150·0 lbs of N_2, 40·0 lbs of P_2O_5 and 80·0 lbs of K_2O per acre . . .. ($N_2P_1K_2$)
(*f*) 150·0 lbs of N_2, 80·0 lbs of P_2O_5 and 40·0 lbs. of K_2O per acre . .. .. ($N_2P_2K_1$)
(*g*) 75 0 lbs of N_2, 40·0 lbs of P_2O_5 and 80·0 lbs of K_2O per acre . .. ($N_1P_1K_2$)
(*h*) 150·0 lbs of N_2, 80·0 lbs. of P_2O_5 and 80·0 lbs. of K_2O per acre .. . .. ($N_2P_2K_2$)

The three fertilizer ingredients, *viz*, nitrogen, phosphoric acid and potash, were added in the form of neutral ammonium sulphate (20·0% N), double superphosphate (40·0% P_2O_5) and potash sulphate (48·0% K_2O), and added in two instalments once after germination and secondly before first earthing the cane.

Experimental Results

No. I Pot Series:

The application of different fertilizers in pots is found to influence the rate of photosynthesis (both apparent and real) in a characteristic way The data obtained give indication to the view that the rate of apparent assimilation increases substantially in response to different treatments

(Fig. 1, Table I). Of the single fertilizer treatment, plants supplied with potash sulphate show the maximum rate of photosynthesis (Expt 4). In the double fertilizer group, plants receiving both nitrogen and potash exhibit the highest photosynthetic rate (Expt 5) followed by those treated with PK and NP (Expts 7 and 6) Plants receiving all three manurial ingredients, however, although assimilate more (Expt. 8) than those receiving PK

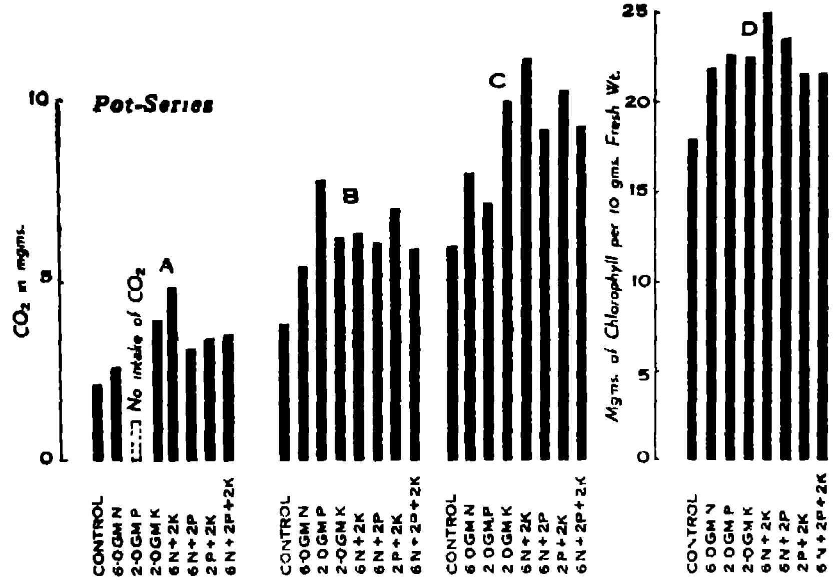

Fig 1

The rate of photosynthesis (apparent and real), respiration and chlorophyll content of potted plants of *Saccharum officinarum*

Pot-Series
A.—Apparent-Assimilation-Rate
B.—Respiration-Rate
C.—Real-Assimilation-Rate
D.—Chlorophyll-Content

(Expt 7) do not show the same high efficiency as that recorded for potash alone (Expt. 4) In response to phosphate in the single fertilizer treated plants there is practically no intake of CO_2 (Expt 3)

The rate of real assimilation varies in practically the same order (Fig 1, Table I) as the rate of apparent assimilation Plants treated with potash alone show greater rate of real assimilation (Expt 4), as compared to nitrogen and phosphoric acid treated plants following in order (Expts 2 and 3). Maximum photosynthetic activity is again noted in plants supplied with nitrogen and potash (Expt 5) In plants receiving all the three fertilizers assimilation neither reaches the level attained by plants treated

TABLE I

Variations in photosynthetic rate (apparent and real), respiration, and chlorophyll content of Saccharum officinarum *in response to different fertilizer treatments*

Temperature = 31° C. ± 0·2

CO_2 concentration = 0·25 to 0·30

Illumination, 1,500 C.P., Half-watt Philips bulb at 18 cm. distance

Expt. No.	Treatment*	CO_2 in mgms. per 100 sq. cm. leaf			Chlorophyll in mgms. per 10 gms. fresh weight
		Apparent assimilation	Respiration	Real assimilation	
	No. 1 Pot series experiments				
1	Control ..	2·171	3·876	6·047	18·0
2	6·0 gms. N per pot ..	2·567	5·455	8·022	21·9
3	2·0 gms. P per pot ..	− 0·515	7·816	7·271	22·5
4	2·0 gms K per pot ..	3·854	6·209	10·063	22·5
5	6·0 gms. N + 2·0 gms. K per pot ..	4·741	6·376	11·117	25·1
6	6·0 gms N + 2·0 gms. P per pot ..	3·156	6·115	9·271	23·5
7	2·0 gms. P + 2·0 gms. K per pot ..	3·322	7·013	10·335	21·7
8	6·0 gms. N + 2·0 gms P + 2·0 gms K per pot .	3·450	5·934	9·384	21·7
	No. 2—Concrete-tank series experiments				
9	Control	2·786	10·511	13·207	15·2
10	Night-soil	2·795	13·429	16·224	16·4
11	Castor-cake	8·540	7·117	15·657	17·5
12	Sheep-dung ..	7·508	9·630	17·138	16·0
13	Cow-dung . .	7·799	8·496	16·295	18·1
14	Compost	7·263	8·928	16·191	18·0

N.B.—The data in these columns are the average of three separate estimations.

* For details of the treatments see pp. 134–36.

TABLE I (*Contd.*)

Expt. No.	Treatment*	CO_2 in mgms. per 100 sq. cm. leaf			Chlorophyll in mgms. per 10 gms. fresh weight
		Apparent assimilation	Respiration	Real assimilation	
	No. 3—Field series experiments				
15	Control	1·191	1·667	2·858	11·25
16	75 N . ..	1·340	4·306	5·646	18·60
17	150 N	3·035	4·155	8·090	22·50
18	40 P . .	2·616	3·139	5·755	18·75
19	80 P	1·556	2·122	3·678	25·00
20	40 K .	6·786	1·068	7·854	13·50
21	80 K	3·998	2·666	6·664	26·0
22	75 N + 40 P	1·419	2·998	4·417	20·00
23	75 N + 80 P	2·449	0·874	3·323	16·60
24	150 N + 40 P	5·571	3·203	8·774	25·00
25	150 N + 80 P ..	3·293	3·911	7·204	25·00
26	75 N + 40 K . ..	1·356	2·304	3·660	25·00
27	75 N + 80 K .. .	(− 1·663)	2·216	0·553	20·00
28	150 N + 40 K .. .	3·877	1·399	5·276	20·00
29	150 N + 80 K ..	3·757	1·878	5·635	25·00
30	40 P + 40 K . ..	2·843	4·314	7·157	26·0
31	40 P + 80 K	6·847	3·423	10·270	26·5
32	80 P + 40 K .. .	7·591	2·137	9·728	22·0
33	80 P + 80 K .	0·864	1·752	2·616	11·2
34	75 N + 40 P + 40 K ..	6·172	10·319	16·491	21·6
35	150 N + 40 P + 40 K .	5·812	1·716	7·555	25·0
36	75 N + 80 P + 40 K .	1·913	2·123	4·336	20·0
37	75 N + 80 P + 80 K .	5·526	1·745	7·271	26·0
38	150 N + 40 P + 80 K ..	7·850	2·685	10·535	18·75
39	150 N + 80 P + 40 K ..	(− 0·636)	1·092	0·720	13·75
40	75 N + 40 P + 80 K .	2·799	3·089	5·888	25·0
41	150 N + 80 P + 80 K ..	1·568	3·051	4·619	23·75

with potash alone or both nitrogen and potash supplied together (Expts. 8, 4 and 5). That the differences under varying fertilizer treatments are highly significant is shown by the results of statistical analysis (Tables II to VI)

TABLE II

Analysis of variance due to artificial fertilizers (Pot Series)

Due to	D F	S.S.	Mean S.S.
Block	2	0·025	0·0125
Treatment	7	100·707	14·3867 V_1
Error	14	2·476	0·177 V_2
Total	23	103·208	

V_1/V_2 = 81·281 (Significant at 1% level). C.D. = 0·686

	6·0 N	2·0 P	2·0 K	6·0 N + 2·0 K	6·0 N + 2·0 P	2·0 P + 2·0 K	6·0 N + 2·0 P + 2·0 K
Control .	+	+	+	+	+	+	+
6·0 N .			+	+	+	+	+
2·0 P .			+	+	+		+
2·0 K .				+			
6·0 N + 2·0 K ..						+	
6·0 N + 2·0 P .						+	
2·0 P + 2·0 K ..							

+ Indicates significant differences

TABLE III

Analysis of variance due to organic fertilizers (Concrete-tank Series)

Due to	D F.	S.S.	Mean S.S.
Block	2	0·515	0·258
Treatment	5	22·931	4·586 V_1
Error	10	15·113	1·511 V_2
Total	17	38·559	

V_1/V_2 = 3·035 (not significant both at 1 and 5%). C.D. = 2·00306.

TABLE III—(*Contd*)

	Night-soil	Castor-cake	Sheep-dung	Cow-dung	Compost
Control ..	+	+	+	+	+
Night-soil ..					
Castor-cake ..			+		
Sheep-dung ..					
Cow-dung ..					

\+ Indicates significant differences.

TABLE IV

Analysis of variance due to single fertilizer series (Field Series)

Due to	D.F.	S.S.	Mean S.S.
Block	2	0·122	0·061
Treatment	6	56·051	9·313 V_1
Error	12	1·473	0·123 V_2
Total	20	57·646	

V_1/V_2 = 75·943 (significant at 1% level)

C.D. = 0·592.

	75 N	150 N	40 P	80 P	40 K	80 K
Control	+	+	+	+	+	+
75 N		+		+	+	+
150 N						
40 P				+	+	+
80 P						
40 K .. .						

\+ Indicates significant differences.

TABLE V

Analysis of variance due to double fertilizer (Field Series)

Due to	D.F.	S.S.	Mean S.S.
Block	2	0·064	0·032
Treatment .	12	36·415	3·034 V_1
Error	24	0·257	0·0171 V_2
Total	38	36·736	

V_1/V_2 = 178·4 highly significant at 1% level. C.D. = 0·214.

	75 N + 40 P	75 N + 80 P	150 N + 40 P	150 N + 80 P	75 N + 40 K	75 N + 80 K	150 N + 40 K	150 N + 80 K	40 P + 40 K	40 P + 80 K	80 P + 40 K	80 P + 80 K
Control	+	+	+	+	+		+	+	+	+	+	
75 N + 40 P			+	+			+	+	+	+	+	
75 N + 80 P			+	+			+	+	+	+		
150 N + 40 P									+	+		
150 N + 80 P									+	+		
75 N + 40 K							+	+	+	+		
75 N + 80 K							+	+	+	+	+	
150 N + 40 K								+	+	+		
150 N + 80 K								+	+	+		
40 P + 40 K												
40 P + 80 K												
80 P + 40 K												

+ Indicates significant differences.

Table VI

Analysis of variance due to three fertilizers (Field Series)

Due to	D.F.	S.S.	Mean S.S.
Block	2	187·807	93·904
Treatment	8	328·587	41·072 V_1
Error	16	126·933	7·933 V_2
Total	26	643·327	

V_1/V_2 = 5·1 significant at 1% level
C.D. = 4·598.

	75 N +40 P +40 K	150 N +40 P +40 K	75 N +80 P +40 K	75 N - 80 P +80 K	150 N +40 P +80 K	150 N +80 P +40 K	75 N +40 P +80 K	150 N +80 P +80 K
Control	+	+		+	+			
75 N + 40 P − 40 K								
150 N + 40 P + 40 K								
75 N + 80 P + 40 K								
75 N + 80 P + 80 K					+			
150 N + 40 P + 80 K								
150 N + 80 P + 40 K								
75 N + 40 P + 80 K								

+ Indicates significant differences.

The rate of respiration is also increased much beyond the value recorded for the untreated plants (Fig. 1) Of all the treatments the rate of respiration is maximum in case of phosphoric acid treated plants (Expt. 3) and minimum in case of plants receiving nitrogen alone (Expt. 2). In other cases the rate of respiration fluctuates within a narrow range

The chlorophyll content of leaves of treated series is always higher than the control (Fig 1) Maximum chlorophyll content is noted in cultures supplied with both nitrogen and potash (Expt 5) followed by those supplied with nitrogen and phosphoric acid (Expt 6).

No II Concrete-Tank Series:

Apparent Assimilation:—The rate of apparent assimilation in response to the organic manures supplied at the rate of 150·0 lbs N_2 per acre, in each case, does not show as characteristic differences (Fig 2) as noted in the

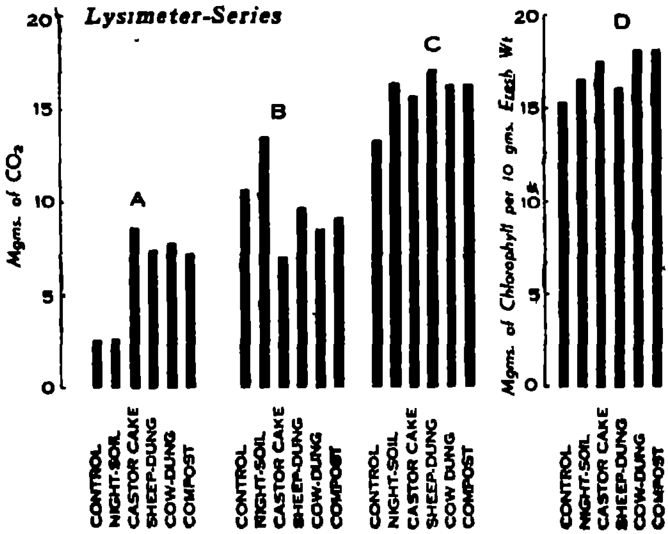

FIG. 2

The rate of photosynthesis (apparent and real), respiration and chlorophyll content of leaves gathered from *Saccharum officinarum* plants growing in concrete tanks

A.—Apparent-Assimilation-Rate
B.—Respiration-Rate
C.—Real-Assimilation-Rate
D.—Chlorophyll-Content

previous case. Leaves from manured plants, however, usually have photosynthetic efficiency much higher than that recorded for the control (Expts. 9–14) In the night-soil treated plants no augmentative effect is, however,

noted (Expt. 10) Castor-cake increases the photosynthetic rate to a maximum extent (Expt 11) followed by leaves from cow-dung, sheep-dung, and compost treated plants (Expts 13, 12 and 14)

Respiration :—While the rate of apparent assimilation is greatly increased in majority of the manures, that of respiration is definitely retarded (Fig. 2) Night-soil, however, increases the rate of respiration (Expt 10) beyond the level attained by the control (Expt 9)

Real Assimilation :—The rate of real assimilation does not vary much from treatment to treatment (Fig 2, Table I) although the treated plants in general show a higher photosynthetic efficiency than the control. The variations due to treatments (Table III) are however not significant

Chlorophyll Content : - Chlorophyll content of leaves too behaves in a similar manner in response to different bulky organic manures (Fig. 2)

These evidences indicate in general that in response to different bulky organic manures (i) the rate of real assimilation and chlorophyll content does not exhibit marked variations from treatment to treatment although the treated plants are definitely better in these regards as compared to control and (ii) the respiratory intensity (night-soil treated plants excepted) is definitely decreased in response to application of organic manures

No III Field Series ·

The rate of assimilation (both apparent and real), respiration and chlorophyll also undergo characteristic variations from treatment to treatment (Figs. 3, 4, 5 and 6 ; Table I)

Apparent Assimilation —The observations indicate that in the single fertilizer series, out of the different treatments given to the plants the greatest acceleration is obtained in plants receiving 40 0 lbs of potash per acre alone (Expt. 20) An increase in the supply of potash to 80 0 lbs. per acre, however, diminishes the rate of apparent assimulation (Expt 21) In contrast to this, however, acceleration is obtained under higher dozes of nitrogen (150·0 lbs per acre) and double superphosphate (80·0 lbs. per acre) (Expts 17 and 19). This indicates that whereas an increase in potash beyond 40·0 lbs. is deleterious in so far as apparent assimilation is concerned, similar increase in nitrogen or phosphates increases photosynthesis markedly

In the 'double fertilizer' series maximum rate of photosynthesis is observed in plants receiving 80·0 lbs of phosphates and 40·0 lbs. of potash per acre (Expt 32, Fig. 3) next in order being those receiving 40·0 lbs. of phosphates and 80 0 lbs of potash per acre (Expt. 31) Plants receiving

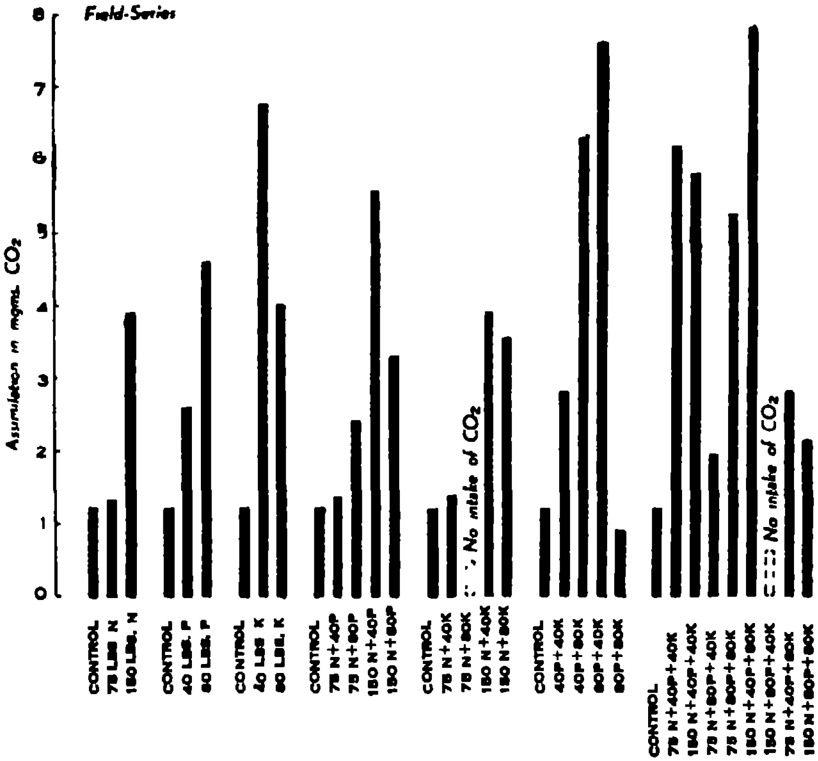

FIG. 3

Fertiliser effect on the apparent assimilation rate of leaves of *Saccharum officinarum* plants

Apparent-Assimilation-Rate

75·0 lbs of nitrogen and 80 0 lbs. of potash do not take up any CO_2 from the atmosphere, the rate of apparent assimilation exhibiting negative values (Expt. 27)

In the three-fertilizer series maximum rate of apparent assimilation is observed in plants receiving 150 0 lbs. of nitrogen, 40 0 lbs of phosphates and 75·0 lbs. of potash (Expt. 38 ; Fig. 3). This is followed by plants grown under $N_1P_1K_1$, $N_2P_1K_1$ and $N_1P_2K_2$ treatments (Expts. 34, 35, 37 ; Fig 3). In this group as well plants receiving 150·0 lbs N_2—80·0 lbs. P_2O_5—40·0 lbs. K_2O per acre exhibit negative values (Expt. 39) there being no intake of CO_2 from the atmosphere

Respiration (*Fig. 4*).—Contrary to the observations recorded for apparent assimilation an increase in potash is associated with increase in respiratory activity (Expts 20 and 21), whereas increase in nitrogen and phosphoric

acid alone to the soil decreases the rate of respiration (Expts 16, 17, 18 and 19).

In the 'single fertilizer' series plants receiving 75·0 lbs. of nitrogen per acre have the maximum rate of respiration (Expt. 16), while those receiving 40·0 lbs. of potash exhibit the least respiration (Expt 20)

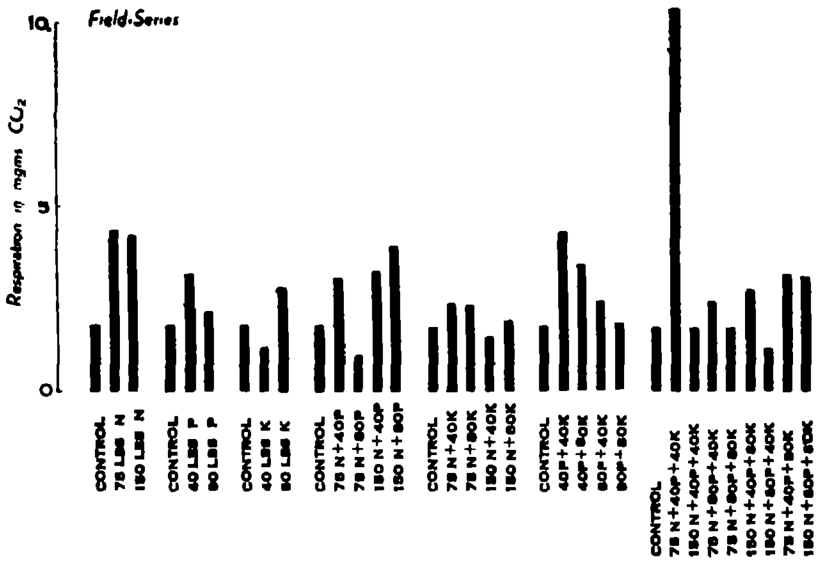

Fig 4

Fertiliser effect on the respiration rate of leaves of *Saccharum officinarum* plants

Respiration-Rate

In the 'double fertilizer' series associations of any two mineral ingredients is useful when applied in certain combination and concentration, whereas in others it is definitely deleterious. Increasing application of phosphoric acid has a deleterious effect on respiration when applied in combination with 75·0 lbs. of nitrogen per acre (Expt 23) Under heavier dressings of nitrogen similar increase in potash increases respiration rate (Expt 29). In combination with phosphorus increase in potash definitely retards respiration at both the levels of phosphoric acid application Maximum rate of respiration is noted in plants receiving both 40·0 lbs of potash and 40·0 lbs. of phosphates per acre (Expt 30). Those receiving 75·0 lbs. of nitrogen and 80·0 lbs of phosphates exhibit least respiration (Expt. 23).

In the 'three fertilizer' series maximum rate of respiration is observed in plants receiving 75·0 lbs N_2—40·0 lbs P_2O_5—40·0 lbs K_2O (Expt. 34)

followed by those grown under $N_2P_2K_2$ and $N_1P_1K_2$ treatments (Expts. 41 and 40). Minimum rate of respiration is observed in plants receiving 150·0 lbs N_2—80·0 lbs. P_2O_5—40·0 lbs. K_2O per acre (Expt. 39)

Real Assimilation (*Fig* 5) —In the ' single fertilizer ' series the rate of real assimilation varies practically in the same order as the rate of apparent assimilation The best result is obtained in case of plants receiving 150·0 lbs. of nitrogen followed by those receiving 40·0 lbs /acre of potash alone (Expts. 17 and 20)

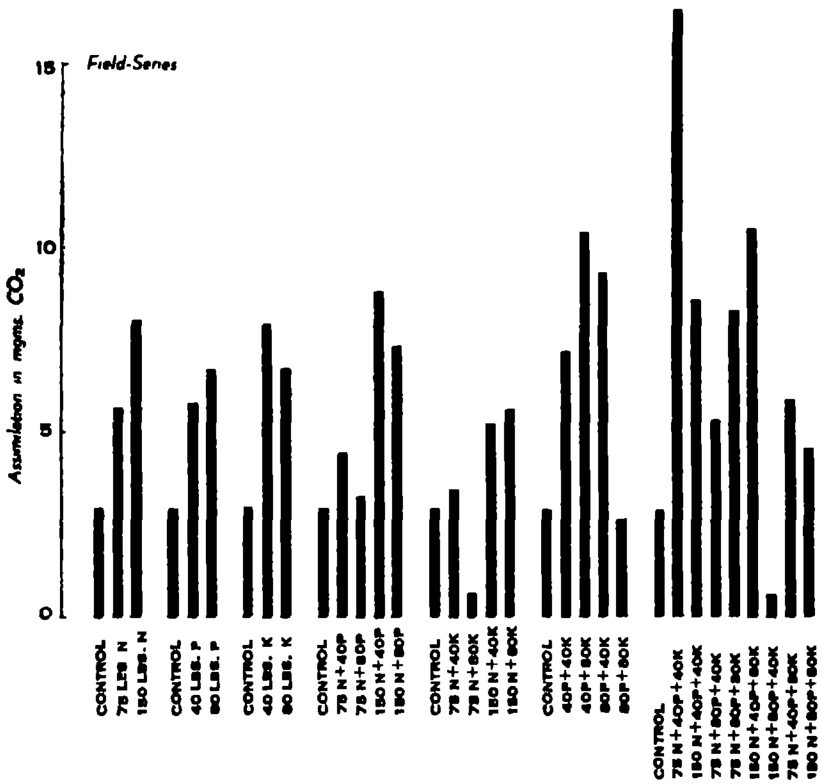

FIG 5

Fertiliser effect on the real photosynthetic rate of *Saccharum officinarum* leaves

Real-Assimilation-Rate

In the ' double fertilizer ' series the maximum rate of real assimilation is observed in plants receiving 40·0 lbs of P_2O_5 and 80·0 lbs of potash (Expt. 31). This is followed by those supplied with 80·0 lbs phosphate and 40·0 lbs potash (Expt 32) Like the rate of apparent assimilation

minimum rate of real assimilation is obtained in plants receiving N_1K_2 treatment (Expt 27)

In association with both higher and lower doses of nitrogen (75·0 lbs and 150·0 lbs per acre) increase in supply of phosphorus decreases photosynthesis (Expts. 23 and 25)

Increase in potash from 40·0 lbs per acre in association with lower doses of nitrogen is much more harmful (Expt. 27) than similar increase in phosphorus (Expt 23)

In association with heavy doses of nitrogen (150 0 lbs per acre), potash when supplied to the extent of 80 0 lbs per acre has a beneficial influence on photosynthesis (Expt 29)

Similar increase in the quantity of potash has a useful influence when applied with 40·0 lbs P_2O_5 per acre (Expt 31) Under heavier doses of P_2O_5 there is, however, a definite harmful effect (Expt 33)

In the 'three fertilizer' series maximum rate of real assimilation is noted in case of plants receiving 75·0 lbs N_2—40·0 lbs P_2O_5—40·0 lbs K_2O per acre (Expt 34) Next in order of efficiency being plants grown under $N_2P_1K_2$ treatment (Expt 38)

The minimum rate of photosynthesis is found in plants receiving 150·0 lbs N_2—80 lbs P_2O_5—40·0 lbs K_2O per acre (Expt 39) The statistical analysis of results (Tables IV VI) indicates that the variation in assimilation rate from treatment to treatment in all the three series are significant at 1% level

Chlorophyll Content (*Fig 6*).—In the 'single fertilizer' series increase in the quantity of either nitrogen, phosphoric acid or potash increases the chlorophyll content of leaves (Expts 15 21) The values in all cases are higher than the control Maximum chlorophyll content is obtained in plants grown under 80·0 lbs of potash followed by those grown under similar doses of phosphates (Expts 21 and 19) In the 'double fertilizer' series increase in phosphorus in association with lower doses of nitrogen appears to be harmful from the point of view of chlorophyll content (Expt 23). In association with heavier dozes of nitrogen of the order of 150·0 lbs. per acre this deleterious influence is overcome, chlorophyll content remaining constant (Expts. 24 and 25)

Increase in potash in association with lower doses of nitrogen has the same effect as phosphorus (Expt 27) When applied with higher doses of nitrogen 150·0 lbs per acre increase in potash decidedly increases the chlorophyll content (Expt 29)

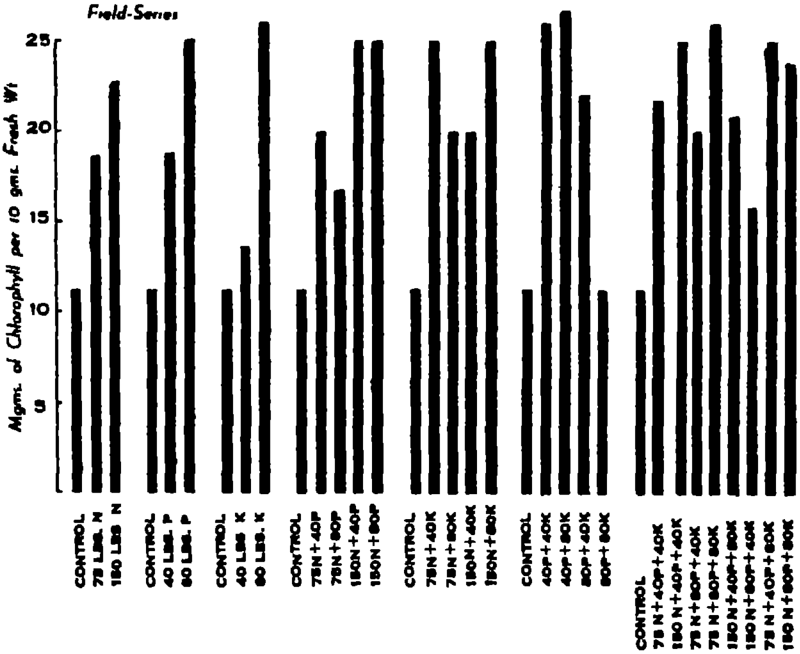

FIG 6
Fertiliser effect on the chlorophyll content
of *Saccharum officinarum* leaves
Chlorophyll-Content

Phosphorus and potash when applied at the rate of 40·0 lbs. and 80·0 lbs. per acre respectively increases the chlorophyll content beyond the values recorded for other combinations of P_2O_5 and K_2O (Expt 31). In association with lower doses of phosphorus increase in potash increases the chlorophyll content (Expt 31) whereas when applied with heavier dressings (80·0 lbs. per acre) of phosphorus similar increase in potash has a definite deleterious effect on the chlorophyll content (Expt 33)

In the 'three fertilizer' series plants grown under $N_1P_2K_2$ treatment (Expt. 37) have the highest chlorophyll content while those receiving $N_2P_2K_1$ treatment possesses the least chlorophyll content (Expt 39). Under other combinations the chlorophyll content varies between 1·8 mgms. to 2·5 mgms. per gm fresh weight.

Discussion

From what has been narrated in the previous pages, it is evident that application of manures either singly or in combination, greatly influences

the rate of assimilation (real and apparent) and respiration as also the chlorophyll content of the leaves It is significant to note that in response to bulky manures (Expts 10–14) such as night-soil, sheep-dung, cow-dung compost, etc, supplied at the rate of 150·0 lbs nitrogen to an acre, photosynthesis does not show well-marked variations from treatment to treatment. On the other hand, application of artificial fertilizer either singly or in different combinations induces wide fluctuations in the photosynthetic efficiency of leaves (Expts 2–8) In response to potassium either applied singly or in combination with nitrogen or phosphorus, photosynthesis is augmented much beyond the value recorded for treatments lacking in potassium

The experiments further reveal that when the quantity of potash is increased from 40·0 lbs to 80·0 lbs. an acre photosynthesis decreases from 7·854 mgms. to 6 664 mgms, *i e.*, 0·85 times (Expts 20 and 21) When a dressing of 75 0 lbs of nitrogen is also supplied with potash, doubling the quantity of potash alone decreases the rate of photosynthesis from 3 660 mgms to 0 553 mgms, *i e*, 0 15 times (Expts 26 and 27) In association with high concentration of nitrogen of the order of 150·0 lbs. an acre the deleterious effect is not evident even when the quantity of potash is increased from 40 0 to 80 0 lbs an acre (Expts 28 and 29) When applied in association with phosphates the photosynthetic response to potash is altogether different In plots supplied with 40 0 lbs of phosphates an increase in potash application from 40·0 lbs to 80 0 lbs increases the rate of assimilation from 7·157 mgms to 10·270 mgms, *i e*, 1 43 times (Expts. 30 and 31) In association with 80·0 lbs of phosphorus similar increase in potash definitely retards the photosynthetic rate by 0·51 times (Expts 32 and 33)

In association with both nitrogen and phosphorus supplied at the rate of 75 0 lbs and 40·0 lbs respectively, increase in potash from 40 0 lbs to 80·0 lbs per acre again decreases photosynthesis from 16 491 to 5·88 mgms, *i e*, 0·36 times (Expts 34 and 40) But with richer dressings of nitrogen of the order of 150·0 lbs of nitrogen, phosphorus being maintained at the same level (40 0 lbs per acre) increase in potash definitely increases the photosynthetic rate from 7 555 mgms to 10·535 mgms, *i e*, 1·39 times (Expts 35 and 38) When phosphorus is also increased side by side with nitrogen upto a level 80·0 lbs and 150·0 lbs respectively, increase in potash by two times again increases photosynthetic rate from 0·729 mgms. to 4·61 mgms., *i e.*, 6 32 times (Expts 39 and 41)

More or less similar variations are observed in response to nitrogen and phosphorus application as well, photosynthesis under certain treatments exhibiting an increase while under others showing a definite decline.

These evidences lead to the generalisation that the photosynthetic response to the application of different manurial ingredients greatly depends upon the presence or absence of other fertilizer ingredients Thus the augmentative effect of potash is marred under certain fertilizer combinations (Expts 27 and 39) when the presence of other complementary factors phosphorus and nitrogen inhibits the fullest acceleration in CO_2 intake No direct proportionality between the quantities of fertilizers applied and the photosynthetic rate is observed in different series This in itself indicates that the response to any of the fertilizers does not rigidly depend upon the concentration of one ingredient alone but differs from treatment to treatment

If the different series of cultures are compared, plants grown under organic manures have higher photosynthetic efficiency than the plants grown in other series not supplied with any superficial dressings of organic matter. Such beneficial effect of organic manures in general might well be attributed to (1) the beneficial effect of organic matter in inducing favourable variations in texture and water-holding capacity of the soil which indirectly influences growth and metabolism, (2) the presence of majority of mineral ingredients essential for normal functioning of the plant machinery, and (3) the presence of the organic nitrogen compounds which have a general stimulating effect upon the plant

Summary

Evidences on the influence of artificial fertilizers and organic manures upon the photosynthetic efficiency, respiration rate, and chlorophyll content of leaves have been discussed in the previous pages The data lead to the conclusion that the influence of potash, phosphorus, and nitrogen upon the photosynthetic activity of leaves greatly depends on the presence or absence of other complementary factors Potash thus when applied singly at the rate of 40 0 lbs per acre increases photosynthesis to a greater extent than heavier dozes (Expts 20 and 21)

In association with both higher and lower doses of nitrogen (75·0 lbs to 150·0 lbs per acre), increase in supply of phosphorus decreases photosynthesis (Expts 23 and 25)

Increase in potash from 40·0 lbs to 80·0 lbs per acre in association with lower doses of nitrogen is much more harmful (Expt 27) than similar increase in phosphorus (Expt 23)

In association with heavy doses of nitrogen (150·0 lbs per acre) potash when supplied even to the extent of 80·0 lbs per acre has a beneficial influence on photosynthesis (Expt 29)

Similar increase in the quantity of potash has a beneficial influence on photosynthesis when applied with 40·0 lbs. of P_2O_5 per acre (Expt. 31); under heavier doses of P_2O_5 there is, however, a definite harmful effect (Expt. 33).

Plants receiving 75·0 lbs of nitrogen and 40·0 lbs. of phosphate and 40·0 lbs potash per acre exhibit maximum photosynthetic rate, next in order being plants receiving 150·0 lbs. of nitrogen, 40·0 lbs. of P_2O_5 and 80·0 lbs. of K_2O per acre

In response to different organic fertilizers the rate of real assimilation does not vary to any significant extent from treatment to treatment, although it is definitely increased beyond the value recorded for the control.

Increasing application of phosphates has a deleterious effect on respiration when applied in combination with 75·0 lbs of nitrogen per acre (Expt. 23). Under heavier dressings of nitrogen similar increase in potash increases respiration rate (Expt 29) In combination with phosphorus increase in potash definitely retards respiration at both the levels of phosphoric acid application (Expts 31 and 32).

Maximum rate of respiration is observed in plants treated with 75·0 lbs. of nitrogen, 40·0 lbs P_2O_5 and 40 0 lbs K_2O (Expt. 34).

Increase in phosphorus in association with lower doses of nitrogen appears to be harmful from the point of view of chlorophyll content (Expt. 23). In association with heavier doses of nitrogen of the order of 150·0 lbs. per acre this deleterious influence is overcome, chlorophyll content (Expts. 24 and 25) remaining constant

Increase in potash in association with lower doses of nitrogen has the same effect as phosphate (Expt. 27) when supplied with higher doses of nitrogen (150·0 lbs per acre) increase in potash decidedly increases the chlorophyll content (Expt 29).

Phosphorus and potash when applied at the rate of 40·0 lbs. and 80·0 lbs. per acre respectively increases the chlorophyll content (Expt. 31) beyond the values recorded for other combinations of P_2O_5 and K_2O. In association with lower doses of phosphorus increase in potash increases the chlorophyll content (Expt. 31) whereas when applied with heavier dressings (80·0 lbs. per acre) of phosphorus similar increase in potash has a definite deleterious effect on the chlorophyll content (Expt 33).

Maximum chlorophyll content is obtained in plants grown under 40·0 lbs. P_2O_5 and 80·0 lbs. K_2O.

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GLUTATHION IN OCULAR DISEASES

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Adams (1925) in her investigations on the crystalline lens found that heat and to a marked extent, ultra-violet rays had a deleterious effect on the glutathion of the lens and that glutathion was absent in lenses in which cataract developed after the administration of naphthalene Gifford (1932) found that some glutathion was always present in such cataractous lenses This difference, the latter author explained, was due to the difference in technic and calculation Bourne and Young (1934) in their work on the metabolism of naphthalene in rabbits showed that naphthalene, while producing cataract, was detoxicated partly by conjugation with cystein forming α-naphthyl mercapturic acid which was excreted in the urine. They suggested that the cataract produced in naphthalene-fed rabbits was probably the result of depriving the lens of cystein Stekol (1935) using dogs as experimental animals also showed that naphthalene was excreted partly as α-naphthyl mercapturic acid in urine. Bourne (1937) mentions in his excellent review on the metabolic factors in cataract production, that, whereas the administration of bromobenzene did not produce cataract, it was excreted in the urine as mercapturic acid, thereby showing that the mechanism of detoxication was the same in the case of naphthalene and bromobenzene Both make use of an –SH compound for conjugation Kirby and R v E-Wemer (1934) in their study on the biology and chemistry of the crystalline lens observed that with the increasing sclerosis of the lens fibres and the decreasing vital activity of the ageing lens, there was a decrease in the concentration of –SH form of glutathion The results of Campbell's investigations (1936) on blood glutathion in individuals with senile cataract and the relation of blood glutathion to sulphur metabolism indicated that there was no marked variation in the reduced glutathion of the blood with age, or diet, or senile cataract even when the amount of sulphur ingested was largely varied

The object of the present investigations was to ascertain if there were any marked variations in the blood glutathion of South Indians having senile cataract, or suffering from other ocular diseases The results of the

investigations are embodied in Table I. The range of variation of blood glutathion is found to be wide The average for reduced glutathion is 25·76 mgm. per 100 c c blood and oxidised glutathion 6·72 mgm per 100 c c. blood. These figures do not differ markedly from the findings of Campbell (1936) for senile individuals having cataract. A statistical comparison of these figures with those of Woodward and Fry (1932) for normal healthy adults shows no significant variation The quotient, GSH/r b c. first introduced by Gabbe (1929) and referred to as Gabbe's quotient in this paper, gives an idea of the glutathion content in unit number of cells. Gabbe's quotient has an average figure of 6·13 When the hæmoglobin values are also incorporated in it (column 13 in Table I) as is done by Woodward and Fry (1932) the average is 5·10 This relative constancy of the modified Gabbe's quotient is in conformity with the observations of Woodward and Fry (1932) in suggesting a compensatory relationship between the hæmoglobin and the blood glutathion or the hæmoglobin may determine that fraction of the glutathion which occurs in the reduced form in the venous blood

In column 4 of Table I is given the habitual diet of the patients According to diet they could be divided into three groups · the ragi eaters, the rice eaters, and the non-vegetarians The rice eaters seem to have the lowest reduced glutathion and the non-vegetarians the highest Gabbe's quotient also shows similar variations while the modified Gabbe's quotient shows the ragi eaters to have the lowest and the non-vegetarians the highest figures

Table II gives the blood glutathion in other ocular diseases The highest values for total glutathion are amongst those with active corneal ulcers with hypopyon The oxidised glutathion has reached the high figure of 26·46 mgm per 100 c c of blood In cases of active corneal ulcers except No 29 the oxidised glutathion is found to exceed the reduced glutathion In other ocular diseases noted in the table the variations in blood glutathion are within the observed limits for normal healthy individuals (*vide* Appendix). Campbell (1936) has found that the range for cases of ocular disease is within the normal But only one case of corneal ulcer is included in that paper

The values of blood glutathion for four normal healthy persons are given in Table III One of these was a girl 11 years of age having the highest reduced and the lowest oxidised glutathion content of blood It would not be safe to draw any inferences from the limited number of cases examined.

Out of the 26 individuals having senile cataract, very few of them show a variation in blood glutathion outside the observed limits for healthy normal persons. The blood glutathion is apparently unaffected by the cataractous

ERRATA

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Page 155, last para, 1st line, *omit* ‘ s ’ in ‘ investigations ’.

Page 156, 1st line, *omit* ‘ s ’ in ‘ investigations ’

Page 157, 1st line, *omit* ‘ s ’ in ‘ inflamations ’

Page 157, 21st line, *for* ‘ metrcially ’, *read* “ metrically ”

Page 159, Table 1, Average in Columns 13 and 14,
instead of ‘ + ’ *read* ‘ F ’.

Page 161, Last two lines under Summary form a separate para

changes in the lens. Only when there is active inflammations of the avascular structures like the cornea the changes in the glutathion of the blood are well marked and they are evidently due to the disease process It may be inferred that the blood glutathion is neither a diagnostic nor an etiological factor in cataract formation.

Experimental

38 Patients, 30 male and 8 female, between 30 and 80 years of age who were under observation in the Krishnarajendra Hospital were chosen for this investigation. 26 of them were having senile cataract and the remaining 12 were suffering from other ocular diseases. Most of them were peasants from the neighbouring villages. A few of them were living in the City and were traders, labourers, or clerks. The blood of each was drawn and analysed individually immediately after drawing it

Glutathion was estimated by the method of Woodward and Fry (1932) in venous blood drawn from the median cubital vein at least two hours after the mid-day meal and at a time when there was no active absorption of lens matter as after preliminary iridectomy or needling

Capillary blood from the finger tips was used for the blood counts and for the estimation of hæmoglobin Blood counts were made in a Thoma counting chamber using Hayem's fluid Hæmoglobin was estimated colorimetrcially in Klett biocolorimeter using a Newcomer hæmoglobin standard disc

In all the determinations the same pipettes set apart and marked for glutathion, red cell counts and hæmoglobin were used The same counting chamber was used throughout

Colour Index was calculated assuming the normal standards as 5×10^6 red cells per mm^3 and hæmoglobin 14 gm per 100 c c blood These figures were chosen because in the several hæmotological studies carried out on Indians by Napier and Das Gupta (1935, 1936) the average figures for healthy normal individuals, have not been below the figures assumed

TABLE I

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Serial No.	Case	Age, sex	Diet	r.b.c. millions/mm.^{3}	Hb gm.%	Colour index	Total GSH mgm./100 c.c.	Reduced GSH mgm./100 c.c.	Oxidised GSH mgm./100 c.c.	Gabbe's quotient	$\frac{\text{Total GSH}}{\text{r.b.c.}}$	Gabbe's Q. × Hb/14	$\frac{\text{Total GSH}}{\text{r.b.c.}} \times \frac{\text{Hb}}{14}$
1	DG	65 m	Ragi	5·30	12·20	0·83	35·87	35·52	0·37	6·70	6·75	5·84	5·88
2	JG	60 m	,,	6·50	12·60	0·70	48·13	42·33	5·80	6·51	7·40	5·86	6·06
3	MG	62 m	,,	5·45	14·60	0·96	34·05	28·83	5·22	5·29	6·25	5·52	6·50
4	GG	65 m	,,	..	..	..	41·10	31·26	9·84	..	..	..	..
5	VG	65 m	,,	2·80	..	..	23·31	20·86	2·45	7·45	8·33	..	..
6	G	55 f	,,	3·96	11·30	0·98	36·54	24·85	11·69	6·25	9·23	5·07	7·45
7	M	60 m	,,	3·74	..	..	33·71	26·07	7·64	6·97	9·01	..	..
8	Ch	55 m	,,	5·80	14·50	0·90	36·80	19·02	17·78	3·28	6·34	3·30	6·39
9	KG	65 m	,,	2·60	8·50	1·02	23·91	21·47	2·44	8·26	9·20	5·01	5·58
10	MG	65 m	,,	3·75	9·80	0·94	22·08	18·40	3·68	4·99	5·88	3·49	4·12
*11	K	70 m	,,	3·80	13·00	1·23	25·76	9·20	16·56	2·42	6·78	2·25	6·29
12	S	60 f	,,	4·60	11·80	0·92	38·34	36·80	1·54	8·20	8·34	6·74	7·03
13	KN	60 f	Rice	3·50	10·60	1·20	30·67	27·61	3·06	7·89	8·76	5·97	6·63
14	NN	60 m	,,	3·20	..	..	22·08	18·40	3·68	5·75	6·90	..	..
15	CC	60 m	,,	4·10	11·00	0·96	28·00	27·00	1·00	6·59	6·83	5·17	5·37
16	NP	60 m	,,	5·50	14·60	0·95	42·01	36·81	5·20	6·69	7·64	6·98	7·96

17	G	65 m	,,	4·76	12·00	0·91	39·26	25·46	13·80	5·30	8·29	4·59	7·09
*18	H	60 f	,,	4·75	11·40	0·86	41·39	11·04	30·35	..	..	..	..
19	NR	65 m	,,	..	..	..	20·24	11·04	9·20	..	..	..	..
20	K	60 f	,,	5·30	..	..	32·20	29·75	2·45	5·61	6·08	..	..
21	P	70 f	,,	5·45	14·20	0·94	32·58	18·71	13·87	3·43	5·98	3·46	6·02
22	DW	60 m	nv	4·75	13·30	1·20	37·51	35·58	1·93	7·49	7·89	7·12	7·50
23	DD	80 m	,,	3·75	10·70	1·03	37·10	33·74	3·36	9·06	9·85	6·84	7·54
24	OS	60 m	,,	5·20	13·00	0·90	30·98	22·24	8·74	4·26	5·96	3·96	5·53
25	SM	80 m	,,	5·40	11·65	0·77	36·20	34·36	1·84	6·29	6·63	5·24	5·51
26	AB	60 m	,,	3·60	9·60	0·96	39·87	23·31	16·56	6·48	11·08	4·44	7·60
Average							33·45	25·76	6·72	6·13 +1·56	7·63	5·10 +1 34	6·46
Range							20·24 48·13	9·20 42·93	0·37 17·78	2·42 9 00	.. .	2·25 6·89	.. ..

m = male ; f = female, nv = non-vegetarian, *oxidised GSH exceeds reduced GSH.

Analysis of Table I (Averages)

Diet	Total GSH in mgm. %	Reduced GSH in mgm %	Oxidised GSH in mgm. %	Gabbe's quotient	$\frac{\text{Total GSH}}{\text{r.b c.}}$	Gabbe's Q. × Hb/ 14	$\frac{\text{Total GSH}}{\text{r.b.c.}} \times \frac{\text{Hb}}{14}$
Ragi .	33·30	26·22	7·08	6·01	7·59	4·79	6·20
nv ..	36·33	29·85	6·49	6·70	7·60	5·52	6·74
Rice .	32·04	22·87	9·18	5·90	7·21	5·23	6·61
Grand Average	33·45	25·76	6·72	6·13	7·63	5·10	6·46

TABLE II

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Serial No.	Case	Age, Sex	Diet	r b c. millions/mm.^{3}	Hb. gm. %	Colour index	Total GSH mgm./100 c c	ReducedGSH mgm./100 c.c	OxidisedGSH mgm.-100 c.c.	Gebbe's quotient	Total GSH / r b.c.	Gabbe's Q × Hb/14	Total GSH / r b.c × Hb/14	Clinical Notes
27	K	50 m	n.v.	3·45	10·40	1·08	33·73	14·41	19·32	4·18	9·78	3·09	7·53	Trachoma with ulcers and leucoma
28	SG	30 m	v	..	..	..	41·10	16·26	24·84	..	..	..	..	Ulcer cornea active
29	Sg	30 m	,,	..	.	..	61·35	40·18	21·17	..	..	..	..	,,
30	C	35 m	,,	5·47	12·30	0·81	39·26	17·79	21·47	3·25	7·18	2·86	6·32	,,
31	S	35 m	,,	5·50	13·90	0·91	19·69	6·55	13·08	1·19	3·58	1·18	3·54	,,
32	M	35 m	,,	5·50	11·10	0·72	28·83	24·53	4·30	4·46	5 24	3·56	4·19	Corneal ulcer healed
33	NP	40 m	,,	4·90	9·00	0·66	25·15	14·41	10·74	2·95	5·13	1·19	3·29	,,
34	MK	70 m	,,	5·88	14·88	0·99	45·42	40·80	4·12	6·77	7·72	7·18	8·18	,,
35	MG	65 m	,,	3·75	15·60	1 49	20·08	18·71	1·37	5·26	5 35	5·89	5·09	Glaucoma
36	G	35 m	,,	5·21	11·11	0·77	25·77	23·31	2·46	4·48	4·95	3 58	3 96	Syphilitic Iritis
37	Tb	30 f	,,	..	..	..	32·60	6·14	26·46	..	.	..	..	Lacrymal abcess with hypopyon ulcer
38	X	64 f	,,	..	..	..	34·97	33·74	1·23	..	..	..	..	Chronic lacrymal infection

TABLE III

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
39	P	11 f	nv	5·08	11·60	0·82	37·42	36·50	0·92	7·19	7·36	5·95	6·10	
40	K	30 m	r.	5·45	..	..	27·80	24·54	3·26	4·50	5·10	..	..	
41	KM	65 m	,,	4·20	12·01	1·02	34·96	32·51	2·45	7·74	8·33	6·64	7·14	
42	M	45 m	,,	4·58	12·60	0·92	33·74	24·55	9·19	5·01	6·88	4·32	5·93	

v = vegetarian, r = rice.

Appendix

Blood glutathion concentration in normal individuals

No. of cases examined	Reduced GSH	Oxidised GSH	Range of normal variation	Author
21	22		11·7–38·8	Schelling, *J. Biol Chem.*, 1932, **96**, 20.
93	27·5		8–16	Bowman, *Proc. Soc Exp Bio. Med*, **31**, 616
30	34	4–11	25–11	Woodward and Fry, *J Biol. Chem*, 1932, **97**, 465.

Summary

The blood of 42 persons, of whom 26 were suffering from senile cataract, 12 from other ocular diseases, and 4 were healthy, has been examined and glutathion, hæmoglobin and red cell count determined in most of the cases and the quotients, GSH/r b c and GSH Hb /r b c calculated The variations in values are within the observed limits for normal healthy individuals
One of us (T P R) desires to record here his thanks to the University of Mysore for the grant of a research scholarship

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ON THE CHARACTERS OF *CHOANEPHORA CUCURBITARUM* THAXTER ON CHILLIES (*CAPSICUM* SPP.)

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Received December 18, 1930

(Communicated by Dr. S. N. Das Gupta)

Choanephora cucurbitarum has been reported (Dastur, 1920) to cause a wet rot of chillies (*Capsicum* sp). In this paper the author has made further observations on the characters of the fungus

Conidia of the fungus growing on the host were inoculated in Brown's standard medium and later monohyphal cultures were obtained to study the characters of the fungus in pure culture

The fungus was identified as *Choanephora cucurbitarum* Thaxter, and compared with its former descriptions of Wolf (1917), Dastur (1920), and Palm and Jochems (1924).

Zygospores

Zygospores developed in culture medium only and not on the host (Text-Fig 11) These are spherical with a thick, smooth and brown epispore and are formed between tips of twining branches (Fig 10) Zygospores measure 55–90μ in diameter.

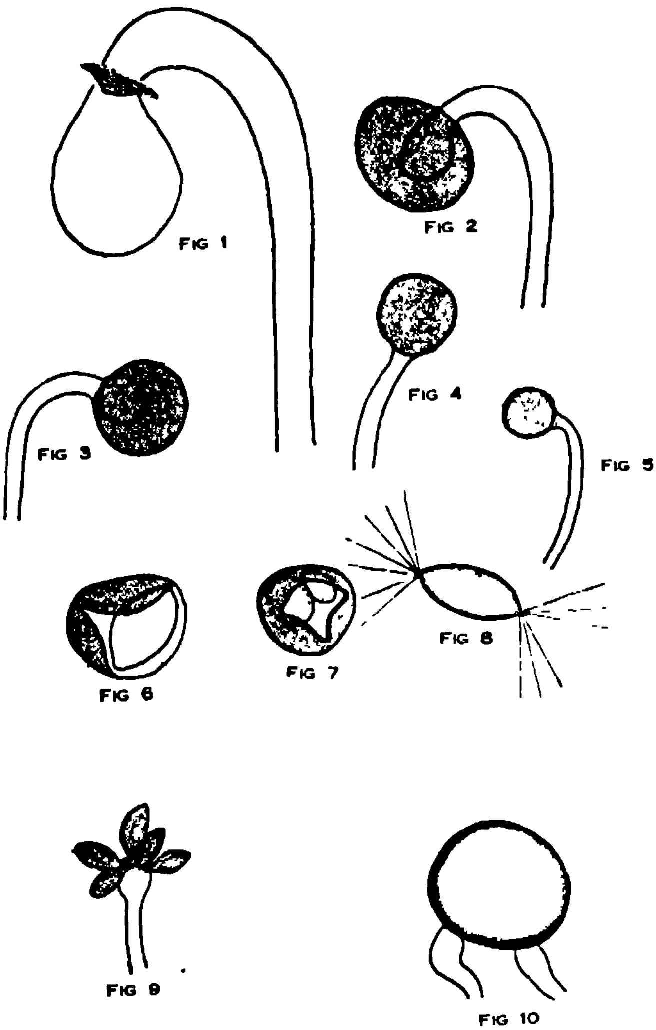
In previous descriptions (1917, 1920) of this fungus it has been stated that the zygospores develop only when the mycelium arises from conidia taken directly from the host and not when conidia are taken from the culture medium During the cultural study by the author no such peculiarity in the formation of zygospores was noticed The zygospores developed from the mycelium arising from the conidia taken directly from the host plant or taken indiscriminately from the fungus growing in the culture medium

Sporangia

The sporangia of this fungus have been observed by Dastur (1920) and Wolf (1917) to develop in culture medium only and never on the host (Text-Fig. 11). Dastur stated that " except conidial stage no other stage of this genus has been yet observed to occur on the host in natural conditions ". But Palm and Jochems (1924) have since reported the occurrence of sporangia of *Choanephora cucurbitarum* on the living plants of *Amarantus Blitum* L. The author also observed the occurrence of sporangia on rotting leaves

and stem of chillies In another paper the author has reported sporangia of *C. cucurbitarum* developing on the plants of *Colocasia antiquorum*.

In nutrient medium Dastur (1920) found that the sporangia of the chilli *Choanephora* were always accompanied by conidia and did not occur apart



FIGS. 1–10. *Choanephora cucurbitarum*

Fig. 1—Sporangiophore with columella, sporangial wall broken and persisting at the base of the columella. × 245. Fig 2—Normal solitary sporangium. Spores not shown. × 245. Fig. 3—Diminutive sporangium with small columella. × 245. Figs. 4 and 5—Diminutive sporangia without columella × 245. Figs. 6 and 7—Diminutive few spored sporangia. × 515. Fig. 8—Sporangiospore. × 515 Fig. 9—Conidiophore—a variation from the normal type. × 245. Fig. 10—Zygospore. × 245 (Drawn under Camera lucida).

from them. But Wolf (1917) has reported the sporangia of *C. cucurbitarum* on squash to occur apart from conidia in culture medium. The author also observed sporangia of the chilli fungus to develop in culture medium both apart from the conidia and in association with them.

In the former description of this fungus on Chillies normal solitary sporangia have been described (Figs 1, 2). Each is terminal and usually pendent on the recurved end of an erect unbranched sporangiophore, provided with a definite columella which tends to become globose and containing a large number of sporangiospores Wolf (1917) has described normal and reduced sporangia from culture medium The diminutive sporangia were as small as 2–3 spored, but it is not stated whether these reduced sporangia had columella or not The author also has observed diminutive sporangia (Figs 3, 4, 5, 6, 7) among the normal ones in culture medium. These reduced sporangia were very small with corresponding reduction in the size of columella which was entirely lacking in still more diminutive ones The number of sporangiospores per sporangium also exhibited a decrease with the reduction in the size of the sporangium In several cases small sporangia without columella and with a single spore have been observed (Fig 6). Palm and Jochems (1924) have suggested that the shape and the size of the columella should be made the basis of classification in determining the species of the genus *Choanephora* as has been done in the genus *Mucor* But the size and also the shape of the columella in *Choanephora* are so variable in one single species of the genus that it would be unsafe to attach much importance to these characters as of specific value

Conidia

The conidial fructifications and conidia are similar to those described by Dastur (1920) But variations from the normal course of development of conidiophores as noted by Wolf (1917) have been found in the chilli fungus as well In culture medium the conidia may arise directly from the surface of primary head (Fig. 9), a condition also noted by Thaxter (1903) and characteristic of the genus *Rhopalomyces* These are not necessarily depauperate forms, since they appear in cultures with normal well-developed fructification.

The observations of the various authors on the occurrence of various spore stages of four species of *Choanephora* have been compared in the text-figure below :

TEXT-FIG. 11

Observations on the occurrence of the various spore stages of the species of Choanephora

Species of the genus	Authors	Conidia		Sporangia		Zygospores	
		On the host	In culture	On the host	In culture	On the host	In culture
Choanephora Simsoni	Cunningham, D. D	−	+	−	+	−	+
Choanephora infundibulifera (Syn. *C. Cunninghamiana*)	Cunningham, D. D.	+	+	−	+	−	+
Choanephora persicaria	Eddy, E. D	−	−	+	+	−	−
Choanephora cucurbitarum (Syn. *C Americana*)	Thaxter, R.	+	+	−	−	−	+
	Wolf, A.	+	+	−	+	−	+
	Dastur, J F.	+	+	−	+	−	+
	Palm, B. T., and Jochems, S C.	+	+	+	+	−	+
	Sinha, S.	+	+	+	+	−	+

Acknowledgment

The author's best thanks are due to Dr S N. Das Gupta for his valuable help and criticism during the course of the investigation

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Fig. 26

Fig. 27

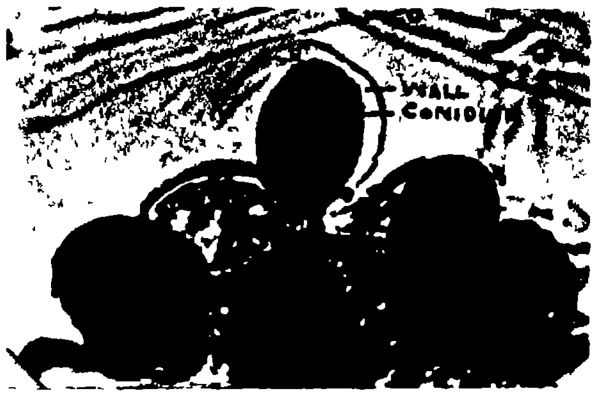

Fig. 28

Fig. 29

Figs. 26-29. *Choanephora trispora*

Fig. 26.—Conidiophore with globose head bearing conidia × 190. Fig. 27. Conidiophore with double head × 190. Figs. 28 and 29. Conidia × 515.

A WET ROT OF LEAVES OF *COLOCASIA ANTIQUORUM* DUE TO SECONDARY INFECTION BY *CHOANEPHORA CUCURBITARUM* THAXTER AND *CHOANEPHORA TRISPORA* THAXTER SP. (=*BLAKESLEA TRISPORA* THAXTER)

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Received December 18, 1939
(Communicated by Dr. S N. Das Gupta)

I *Introduction*

DURING the rainy season of 1938 the leaves of *Colocasia antiquorum* were found to suffer from a pulpy rot which appeared to differ greatly from the 'blight' disease caused by *Phytophthora Colocasiæ* On examination the rotten leaves were found to be affected with *Phytophthora Colocasiæ* and two other fungi which have been identified as *Choanephora cucurbitarum* Thaxter, and *Choanephora trispora* Thaxter sp. (= *Blakeslea Trispora* Thaxter). These two latter fungi occurred either separately or in combination with *Phytophthora Colocasiæ* Several fields in the vicinity of Lucknow were visited and large amount of the material was collected and examined periodically.

II. *Symptoms*

The disease first shows itself on the leaves, the earliest attack occurring in August, in the production of conidial and sporangial fructifications in the lesions caused by *Phytophthora Colocasiæ* The affected areas advance further irregularly presenting water soaked character where the pathogens grow luxuriantly and before long the lamina falls in bits In severe cases of attack the petiole also becomes affected and the whole lamina, due to its weight, falls to the ground as a rotten pulpy mass The pulpy character of the rot distinguishes it from the blight disease and overshadows the symptoms produced by *Phytophthora Colocasiæ* The disease is first perceptible when crops of sporangial and conidial fructifications have appeared As already observed by Cunningham (1879) for *Choanephora infundibulifera* and by Möller (1901) for *Choanephora cucurbitarum* the spore bearing fructifications develop only at night or during the very early hours of morning In the afternoon and evening these were produced sparingly High humidity especially favours

the incidence and spread of the disease which becomes evident in fields from early August to late October.

III. Causal Agents

The two fungi isolated were identified as *Choanephora cucurbitarum* Thaxter and *Choanephora trispora* Thaxter sp. (= *Blakeslea trispora* Thaxter) and compared with former descriptions given by Thaxter (1914), Wolf (1917), Dastur (1920), Palm and Jochems (1924) and Zycha (1935).

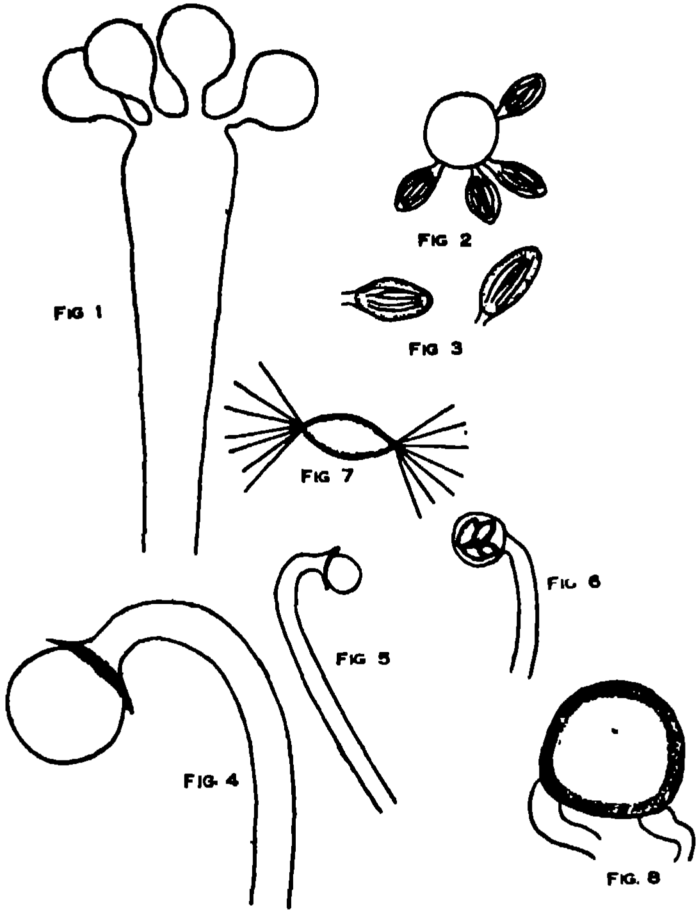
IV. Morphology of the Pathogens

A. Choanephora cucurbitarum Thaxter.

The fungus was isolated from affected leaves. The conidia from the host were inoculated in standard synthetic medium (Horne and Mitter) having the composition: $MgSO_4$ $7H_2O$, 0·75 gm, Asparagin 2 gm, K_2PO_4 1·25 gm, Glucose 2 gm Potato Starch 10 gm, Agar 15 gm, and water 1,000 c c To ensure the purity of the strain monohyphal cultures were made The characters described for the fungus relate to those obtained both from the original strain growing on the diseased tissue as well as those found in monohyphal cultures

Conidia —These occur on the host and also develop in culture medium. The conidiophore (Fig 1) is erect ending in a capitate vesicle from which a few short branches emerge, which in turn become vesicular. The latter at maturity are covered with conidia The conidia (Figs 2, 3) are ovoid, non-ciliate, longitudinally striate and often provided at the base with a short hyaline stalk These measure 15·6–24 × 10–13 μ in size

Sporangia —These occur on the host and also develop in culture medium. The sporangium (Fig 4) is terminal and usually pendent on the recurved end of an erect sporangiophore, provided with a definite columella which tends to become globose The sporangium contains a large number of sporangiospores. Its wall is tuberculate In Dastur's (1920) account the wall has been described smooth but Möller (1901) and Palm and Jochems (1924) describe the wall finely sown with minute granulations Diminutive few spored sporangia (Figs. 5, 6) occur in culture medium These show a gradual reduction in the size of columella and in the number of spores In the very much reduced sporangia the columella is lacking and the spores are 2–3 in number The sporangia measure 25–175 μ in diameter The sporangiospores (Fig 7) are ovoid, smooth and not striate like conidia and are provided at both ends with a cluster of fine radiating appendages. The spores are 15–22 × 8–13μ in size.



FIGS 1–8. *Choanephora cucurbitarum*

Fig. 1—Conidiophore with secondary heads. ×215. Fig 2 Conidial head bearing conidia ×390 Fig. 3—Conidia. ×515. Fig 4—Sporangiophore with columella, broken wall of sporangium persisting at the base ×245 Figs. 5 and 6 Diminutive sporangia. ×245 Fig. 7—Sporangiospore. ×515. Fig 8 Zygospore. ×245.

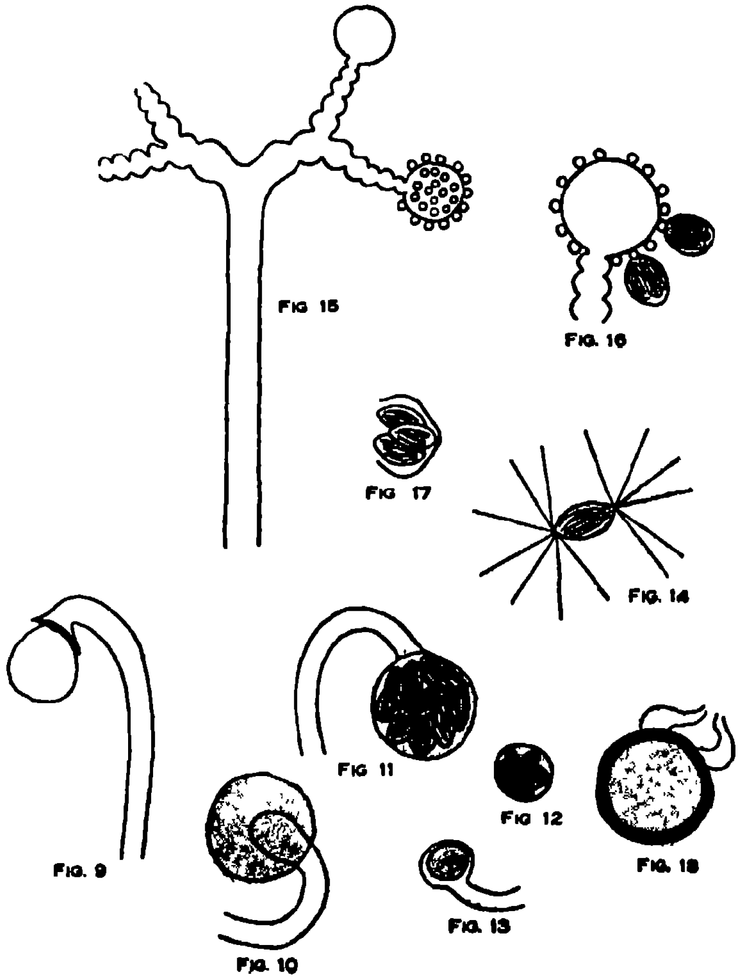
Zygospores —These develop in culture medium and not on the host The zygospores are formed between tips of twining branches and measure 54·3–89·6μ in diameter (Fig 8)

B. Choanephora trispora Thaxter sp (= *Blakeslea trispora* Thaxter):

The fungus was isolated from the diseased leaves. For cultivation in the nutrient medium sporangiospores from the host were inoculated in the standard medium. Monohyphal cultures were obtained to ensure the purity of the strain. The characters of the fungus described here are those observed

in the material from the host tissue and from that growing in the monohyphal cultures.

Sporangia —The sporangial fructifications occur abundantly on the host and also develop in culture medium The sporangia are large, solitary, spherical and terminal on the recurved end of the sporangiophore (Figs. 9, 10). It possesses a large columella hemispherical to elongate in shape and



FIGS 9–18. *Choanephora trispora*

Fig. 9—Sporangiophore with a columella, broken wall of sporangium persisting at the base of columella. ×84. Fig. 10—A normal solitary sporangium; spores not shown. ×245. Figs. 11, 12 and 13—Diminutive sporangia. ×390, ×245, ×390. Fig. 14—Sporangiospore. ×515. Fig. 15—Sporangiophore with sporangioliferous heads. ×245. Fig. 16—Sporangioliferous head bearing sporangiola. ×390. Fig. 17—A three-spored sporangiolum. ×515. Fig. 18—Zygospore. ×245.

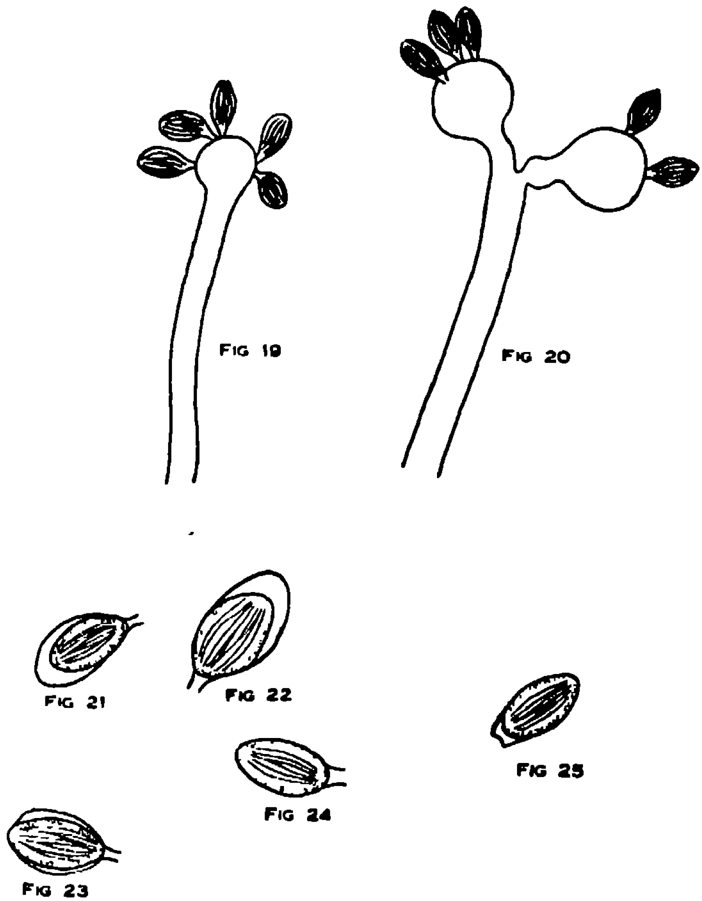
containing a large number of spores The sporangial wall is coarsely roughened as is also the distal end of the sporangiophore. In culture medium small terminal reduced sporangia (Figs 11, 12, 13) were also formed. These possess a small columella which gradually disappear in very diminutive ones. The number of sporangiospores per sporangium becomes reduced in relation to the size of the sporangium In extreme cases of reduction small sporangia with 1–2 spores (Fig 13) have been found. The solitary sporangia measure 17·7–185·5 μ in diameter The spores are variable in size 8–13 μ × 5–6 μ, brown, longitudinally striate and provided at each end with a cluster of fine radiating appendages (Fig. 14).

Sporangiola —The sporangiola are formed on the host and also in culture medium. Each resembles a small solitary sporangium but without a columella. The sporangiola occur in considerable number over the surface of large sporangioliferous heads which are usually in groups of ten or more, terminating on the branchlets of the subdichotomously branched sporangiophores (Fig 15) The sporangiolum is typically three spored, rarely four spored, attached to the sporangioliferous head by a small spherical vesicle (Figs 16, 17) A single sporangiolum measures 13–15 μ in diameter The spores are like those of solitary sporangia

Conidia —Conidial fructifications have not been observed as yet to occur on the host These were found only in nutrient medium The sporangiospores from the host were inoculated in Horne and Mitter's medium and later a monohyphal culture was made in the same medium At the fourth generation (subculture) the fungus was transferred to potato-agar medium While examining the fungus at the second subculture in the potato-agar medium it was found that conidial fructifications had developed in association with sporangial fructifications

Each conidiophore terminates in a single globose head (Figs 19, 26) or sometimes a double head may be formed (Figs 20, 27) The conidia are borne on these heads on short stalks. The conidia are of two types both occurring indiscriminately on the same conidial head The one type of conidia (Fig 24) resembles those of *Choanephora cucurbitarum* These are dark brown, longitudinally striate and measure 23–27 μ × 8–13 μ The other type of conidia (Figs 21, 22, 23) and (Figs 28, 29) is of much interest. These are similar to the conidia described above but are enclosed in a colourless wall to a varying extent. While making glycerine preparations it was observed that the enclosing wall sometimes separated off the conidium showing that the wall is mechanically separable. In *Choanephora cucurbitarum* there are some conidia (Fig. 25) whose basal end is just enclosed

by a narrow hyaline wall This led Thaxter (1914) to suggest a homology between the conidia of *Choanephora cucurbitarum* and the sporangiola of



FIGS. 19–25. *Choanephora trispora*

Fig 19, 26—Conidiophore with globose head bearing conidia ×245. Fig. 20, 27—A conidiophore with double head ×245. Figs 21, 22, 23, 24, 28, 29—Conidia. ×515 Fig. 25—Conidium of *Choanephora cucurbitarum*. ×515

(All the figures have been made by the help of a Camera lucida.)

Blakeslea According to him the conidia are " monosporous sporangiola ". This view is confirmed by the present observations. The second type of conidium described above is enclosed in a wall and is evidently a " monosporous sporangiolum " with one spore which is striate but without the cluster of appendages at the ends The conidium seems to have thus formed by the fusion of the original sporangial wall with the spore.

Zygospores.—These develop only in culture medium and formed between the tips of twining branches (Fig. 18), measuring 50–60 μ in diameter

V. Discussion

The wet rot of *Colocasia antiquorum* is caused by two fungi, *C cucurbitarum* Thaxter and *C trispora* Thaxter sp (= *Blakeslea trispora*) It will be seen from the description given above that *C trispora* resembles *Blakeslea trispora* in all details except for the fact that *C trispora* possesses conidia which are not found in *B trispora* In fact it is the two characters, *i.e.*, the absence of conidia and the presence of sporangiola, which distinguishes the genus *Blakeslea* from the genus *Choanephora* A comparison of all the available records of species of *Choanephora* with *B trispora* will bring out the point clearly (Fig. 30) It will be seen that all the species of *Choanephora* except *C. persicaria* possess conidia, sporangia and zygospores but the occurrence of sporangiola is altogether lacking except in *C trispora* now described *C trispora* has the characters both of *Choanephora* and *Blakeslea* in that it produces conidia like those of *Choanephora* and sporangiola as in

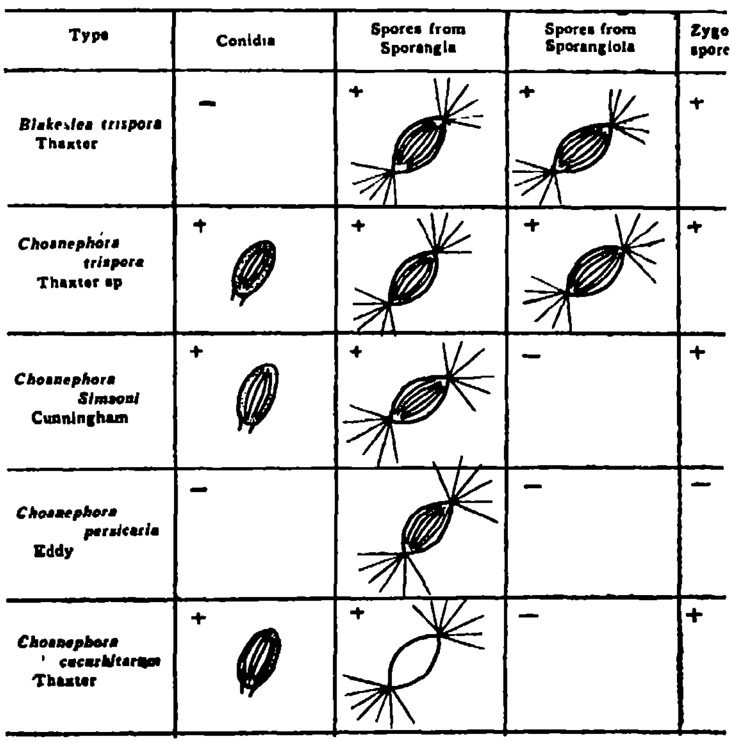


FIG. 30

Spore Characters of species of *Choanephora* and *Blakeslea* compared

Blakeslea, the sporangial stage being common to all. Further the conidia observed in *C trispora* are similar to those described for *C cucurbitarum* and *C Simsoni* The sporangia and the spores in *C trispora* are similar to those of *B trispora*, *C Simsoni* and *C persicaria* The sporangiola and the sporangiospores are exactly alike in *C trispora* and *B trispora* The spores from the sporangia and sporangiola in *B trispora* are exactly similar to those from sporangia in *C trispora*, *C Simsoni* and *C. persicaria* and also to the spores of sporangiola in *C trispora*

A consideration of all these facts will show that the fungus (*C. trispora*) described here may be regarded as a species of *Choanephora* It is proposed therefore to abolish the genus *Blakeslea* and rename *B trispora* as *Choanephora trispora*

The striking similarity between *Blakeslea* and *Choanephora* was long pointed out by Thaxter (1914) and the generic separation of the two forms was doubted by him As already stated the separation was based on the absence of conidia and presence of sporangiola. The presence of conidia in *C trispora* removes this distinction between the two genera The presence of sporangiola in *C trispora* does not seem to be a character of sufficient importance to include the fungus in *Blakeslea* and retain the genus. Particularly Thaxter (1914) states to have observed structures in culture medium showing "almost every imaginable intermediate condition between solitary sporangia and sporangiola" Presence of such transitional stages between sporangia and sporangiola makes the latter of doubtful systematic value particularly for generic separation

The types of conidia observed in *C trispora* seem to be of special interest inasmuch as these forms are definitely "monosporous sporangiola" and form intermediate structures in the derivation of conidia from sporangiola. Such a transition was pointed out by Thaxter (1914) who particularly emphasised the close correspondence between the conidial fructification of *C cucurbitarum* and sporangioliferous fructifications of *B trispora*. A still closer correspondence exists in *C dichotoma* in which the conidiophore is dichotomously branched like the sporangiophore of a sporangioliferous fructification

The occurrence of *C cucurbitarum* and *C trispora*, the wet rot fungi, in the lesion caused by *Phytophthora Colocasiæ* indicates that the fungi are incapable of attacking a normal healthy host but can behave as facultative parasites once they get entry and establish themselves in the host and produce further rot independently of *Phytophthora Colocasiæ* Thus the fungi

which first appear as saprophytes subsequently behave as weak parasites The nature of weak parasitism of the members of Choanephoraceæ has been mentioned by several authors Wolf (1917), Dastur (1920), Palm and Jochems (1924) and Eddy (1925) The capability of these fungi to behave as parasites has been of much interest to plant pathologists since these cause decay of fruits and vegetables in storage and transit.

VI. Summary

1. A wet rot of leaves of *Colocasia antiquorum* has been reported to be caused due to secondary infection by *Choanephora cucurbitarum* Thaxter and *Choanephora trispora* Thaxter sp. (= *Blakeslea trispora*). The disease appears during the months of August-October on plants already affected with *Phytophthora Colocasiæ* The pathogens appear as saprophytes in the lesions caused by *Phytophthora Colocasiæ* and produce further rot behaving as facultative parasites.

2. The morphological characters of the fungi have been described. The species previously described as *Blakeslea trispora* Thaxter is now transferred to the genus *Choanephora* and reasons are given for abolition of the genus *Blakeslea* of which no other species is yet known

3. Conidia enclosed in a wall resembling "monosporous sporangiola" have been found in culture medium in *Choanephora trispora* These 'monosporous sporangiola' have been regarded as intermediate structures illustrating the derivation of conidia from sporangiola

Acknowledgments

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MITES OF THE FAMILY TETRANYCHIDÆ FROM LYALLPUR WITH DESCRIPTIONS OF FOUR NEW SPECIES

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This paper is based on the phytophagous mites collected at Lyallpur In all seven species are described of which four are new to science. Biological notes are given for each species

De Faur's fluid (Imms, 1929)[1], Berlese fluid (Imms, 1929)[1] and chloral hydrate (saturated solution) were tried to kill the mites, but they made the chitin weak and crumpy Ultimately a solution containing 50 c.c. of water, 50 c.c of glycerine, and 1 0 gram of hydrochloride of cocain was prepared for the purpose and was found to be satisfactory in every way

"Irrigation" method was employed in treating the mites with the reagents usually employed in preparing canada-balsam mounts. Specimens were stained with picric acid (saturated solution in 90% alcohol). 575 times magnification was employed to sketch such structures as palps, penis, collar, trachea and claws.

Mites were killed with the fumes of glacial acetic acid for studying their chætotaxy and sketches made at 103 times magnification.

Living specimens were used for describing the coloration.

Key to Genera

The species described in this paper are contained in three genera, which can be identified with the following key :—

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[1] Imms, A. D., *Bull. Ent. Res.*, 1929, **20**, 165–71.

1. (2) Claws of legs absent (Fig. 6*e*) .. Genus : Anychus (McGregor)
2. (1) Claws of legs present
3. (4) Claws of legs always simple and entire, neither bifurcated nor furnished with teeth In male, claws of anterior pairs of legs similar and without hairs on protuberances (Figs. 4 *f* and 4 *g*), claws of posterior pairs of legs also similar but with hairs on protuberances (Fig 4 *h*) In female, claws of all legs similar and with hairs on protuberances.. Genus · Paratetranychus (Zacher)
4. (3) In male, claws of first legs either ending in minute teeth (Figs 1 *e* and 3 *e*) or bifurcated (Fig 2 *e*), claws of other legs either ending in hairs (Fig 1 *f*) or bifurcated. In female, claws of all legs similar, either ending in hairs or bifurcated . Genus . Tetranychus (Dufour)

Hirst (1920)[2] has based the classification of the species on the shape and size of the finger-like projections on the *thumb of the palps* and *claws of the tarsi* in both sexes and the *shape of penis* in male He disregards *dorsal chætotaxy, mandibular plate, collar trachea* and *legs* which, according to McGregor (1935)[3] afford important additional diagonostic characters We have taken these characters into consideration and have described the species accordingly.

KEY TO SPECIES OF GENUS TETRANYCHUS

1. (2) Claws of the first leg in male bifurcated (Fig 2 *e*) .. . *Tetranychus mori* sp nov.
2 (1) Claws of the first leg in male with teeth arranged in a whorl (Fig 1 *e* and 3 *e*) .. .. ..

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[2] Hirst, S., *Proc. Zool. Soc. London*, 1920, pp. 49–60.

[3] McGregor, E. A., *Proc. Ent. Soc., Washington*, 1935, 37, 101–05.

3. (4) Cone of thumb of male absent (Fig. 3 *a*) .. .. *Tetranychus fici* Hirst.
4. (3) Cone of thumb of male present (Fig. 1 *a* and 1 *g*)
5. (6) Penis broadly triangular with distal end curved (Fig 1 *i*) .. .. *Tetranychus telarius* L.
6. (5) Penis more or less cylindrical with the distal end straight (Fig 1 *c*) .. *Tetranychus cucurbitæ* sp nov

Tetranychus cucurbitæ sp nov.

(Fig 1, 1 *a*, 1 *b*, 1 *c*, 1 *d*, 1 *f*, 3 *e*)

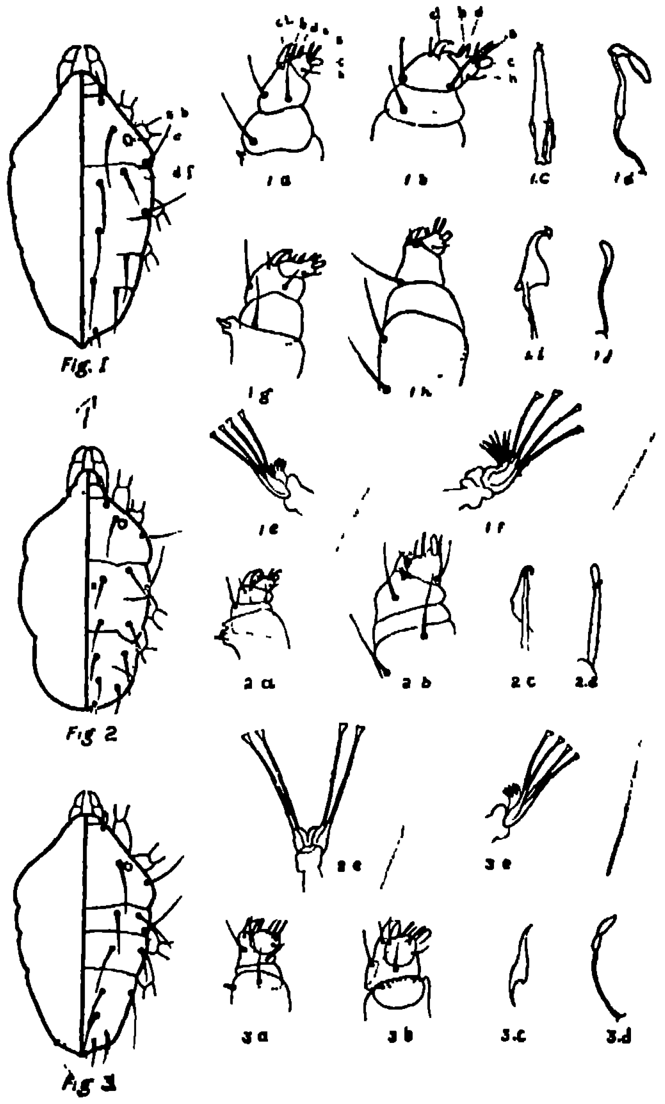
Female ·

Length (without palps) on slide . 0·37 -0 50 mm

Breadth on slide .–0 26–0·37 mm (broadest on either side of the dorsal furrow).

Segmentation of body distinct and with four transverse lines on dorsum, posterior three lines distinct only along the margins (Fig 1) *Coloration* very variable, greyish green to brown, light-brown specimens most common, cephalothorax and appendages lighter in shade, dorso-latrum with dark specks which increase with age and finally cover the entire dorsum. *Chæto-taxy* (Fig 1) : dorsum with 12 pairs of hairs, pair on either side of apex of abdomen minute and without annular base, third and fourth pairs with the dorsal-furrow (Fig. 1, *d. f*) in between, sub-frontal pair (Fig. 1, *s. b.*) well ahead of the line joining anterior margins of the eyes

Eyes (Fig. 1 *e*) · deep red, irregular and single-corneated *Mandibular plate* without anterior notch, length to breadth ratio 35 20 *Collar trachea* (Fig. 1 *d*) : elbowed with undivided distal cell, *Palps* (Fig. 1 *b*) fifth segment with three hairs, one near base of claw smallest , *thumb* with a hair (Fig 1 *b*, *h*) and a bristle (Fig 1 *b*), *cone* (Fig 1 *b*, *c*) finger like, slightly longer than broad, length and breadth being 4·0 μ and 3 0 μ respectively, and equal in length to dorsal finger (Fig 1 *b*, *d*) which is pointed distally, two sensillæ (Fig. 1 *b*, *s*) 7·0 and 5·0 μ in length, longer inserted close to base of cone Claw (Fig. 1 *b*, *cl*) moderately developed but strongly curved *Legs* : first pair longest, fourth pair shorter than first but longer than second and third, which are equal (Statement I).



Tetranychus cucurbitæ sp. nov.

FIG. 1.—Showing shape and arrangement of hairs on dorsum of female (*s.b.*, subfrontal bristle; *e*, eyes; *d f.*, dorsal furrow). 1 *a*.—palp of male (*h.*, hair; *c.*, cone; *s.*, sensillæ; *d*, dorsal finger; *b.*, bristle; *cl.*, claw). 1 *b*.—palp of female (*h.*, hair; *c.*, cone; *s.*, sensillæ; *d.*, dorsal finger; *b.*, bristle; *cl.*, claw). 1*c*.—penis. 1*d*.—collar trachea.

Tetranychus telarius L. 1 *e*.—claw of first leg of male. 1 *f*.—claw of legs of female 1 *g*.—palp of male. 1*h*.—palp of female. 1*i*.—penis. 1*j*.—collar trachea.

Tetranychus mori sp. nov.

FIG. 2.—Showing shape and arrangement of hairs on dorsum of female. 2 *a*.—palp of male. 2 *b*.—palp of female. 2 *c*.—penis. 2 *d*.—collar trachea. 2 *e*.—claw of first leg of male.

Tetranychus fici Hirst

FIG. 3.—Showing shape and arrangement of hairs on dorsum of female. 3*a*.—palp of male. 3 *b*.—palp of female. 3 *c*.—penis. 3 *d*.—collar trachea. 3 *e*.—claw of first leg of male.

STATEMENT I

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·04	0·04	0·10	0·06	0·07	0·07
II & III	0·04	0·03	0·08	0·05	0·06	0·05
IV	0·04	0·04	0·08	0·06	0·07	0·06

Claws of all legs similar and split into six hair-like processes (Fig 1 *f*) Tennent hairs four (Fig 1 *f*)

Male:

Length (without palps) on slide. 0·30–0·40 mm

Breadth on slide: 0·18–0·23 mm. (broadest at the posterior margin of cephalothorax)

Segmentation like that of female, but all lines distinct dorsally. *Coloration*: light brown to brown, appendages and cephalothorax comparatively lighter, dorsum usually without dark specks, older specimens may, however, have a few of them along the mid-dorsal line. *Chætotaxy* similar to female.

Eyes, mandibular plate and collar trachea like those of female *Palps* (Fig 1 *a*): fifth segment with two long hairs, '*thumb*' with one hair (Fig 1 *a*, *h*) on inner side below cone and a bristle (Fig 1*b*) between claw and dorsal finger; *cone* (terminal finger) (Fig 1*a*, *c*): suboblong 6·0 μ long, being twice its breadth and equal in length to shorter of the sensillæ (Fig 1 *a*, *s*), dorsal finger (Fig. 1 *a*, *d*) 4·0 μ and other sensilla 7·5 μ in length Claw (Fig 1 *a*, *cl*) of moderate size but weakly curved *Legs*: first pair longest, fourth pair shorter than first but longer than second and third, which are equal (Statement II).

STATEMENT II

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·05	0 03	0·09	0·05	0·06	0·07
II & III	0·04	0·02	0·08	0·04	0·04	0·06
IV	0·04	0·02	0·07	0 05	0·05	0·06

Claws of the first leg (Fig 3 *e*) ending in a whorl of teeth but those of others (Fig 1 *f*), like those of female, split into six hair-like processes Tennent hairs four (Fig 1 *f*)

Penis (Fig 1 *c*) 36 0μ long, strongly chitinised more or less cylindrical but slightly swollen towards the distal end which bears two distinct barbs.

Biological notes —*Tetranychus cucurbitæ* has been recorded from about sixty different kinds of plants, but 'tinda' (*Citrullus vulgaris* var *fistulosus*); pumpkins (*Curcubita maxima, C pepo,* and *C moschata*) ; 'ghia-tori' (*Luffa aegyptica*) ; cabbage (*Brassica oleracea*) ; tomato (*Lycopersicum esculentum*) ; and hollyhock (*Althaea rosea*) are, however, more seriously infested It covers with web the entire leaf which gradually dries and ultimately drops off the plant Its attack is at its maximum during April–June.

It winters usually as gravid females, a few of which oviposit whenever the season warms up a bit General oviposition, however, starts about the end of February or beginning of March and by the end of April it is found on a majority of its host-plants Monsoon rains kill all stages except the eggs. The progeny from these eggs starts fresh infestation It again becomes active during September–October

Tetranchus telarius L

(Figs. 1 *e*, 1 *g*, 1 *h*, 1 *i*, 1 *j*)

This species was described by Hirst (1920)[4], but he did not take into consideration ***dorsal chætotaxy, mandibular plate, collar trachea*** and ***legs***. It is, therefore, redescribed here.

[4] Hirst, S., *Pro. Zool. Soc. London*, 1920, 49–60.

***Female*:**

Length (without palps) on slide: 0·36–0·50 mm

Breadth on slide: 0·28–0·36 mm (broadest on either side of dorsal furrow).

Segmentation like that of *T cucurbitæ* *Coloration* of two distinct types, light brown and pink, light brown specimens with dark specks. Dorsal chætotaxy also identical with *T cucurbitæ* (Fig 1)

Eyes red, irregular in outline and single-corneated *Mandibular plate* without anterior notch, length to breadth ratio 31 20 *Collar trachæ* (Fig 1 *j*) more or less straight with distal cell undivided and club-like *Palps* (Fig 1 *h*) fifth segment with three hairs, two short and located near base of claw, '*thumb*' with one hair arising below cone, cone 4·0 μ long and 2 5 μ broad; dorsal finger as long as breadth of cone, sensillæ equal in length each being 5·0 μ Claw moderately developed but broad at base *Legs*. first pair longest, fourth pair shorter than first but longer than second and third which are equal (Statement III).

STATEMENT III

Showing Length (in mm.) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·05	0·03	0 12	0·055	0·075	0 11
II & III	0·04	0·025	0 08	0 050	0·06	0·08
IV	0·04	0 025	0·10	0·055	0 065	0·10

Claws of legs (Fig 1 *f*) similar to those of legs to *T cucurbitæ* female

Male:

Length (without palps) on slide. 0 30–0 35 mm

Breadth on slide · 0·17–0·21 mm (broadest at the posterior margin of the cephalothorax)

Segmentation and Dorsal chætotaxy like those of *T cucurbitæ*. *Coloration* of two distinct types, light brown and pink

Eyes, *mandibular plate* and *collar trachæ* like those of female. *Palps* (Fig 1*g*): 5th segment with three hairs, smallest arising near base of claw;

thumb with a hair and two bristles, between claw and dorsal finger ; *cone* 4·0 μ long, 1·5 μ broad and equal to two sensillæ, dorsal finger 2·5 μ in length *Legs*—1st pair longest, 4th shorter than 1st but longer than 2nd and 3rd, which are equal (Statement IV)

STATEMENT IV

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·04	0·025	0·10	0·045	0·06	0·09
II & III	0·03	0·02	0·07	0·03	0·05	0·06
IV	0·03	0·02	0·08	0·045	0·055	0·09

Claws of the first leg with a dorsal tooth and a whorl of teeth (Fig. 1 *e*) ; dorsal tooth absent in posterior legs.

Penis 18·0 μ long (Fig. 1 *i*) more or less triangular with apex curved which is furnished with two minute barbs.

Biological notes —*Tetranychus telarius* is a serious pest of a large majority of plants and is often found with *T cucurbitæ* Red specimens, which become very common from July onwards, however, are always found alone on such plants as lady's finger (*Hibiscus esculentus*), " tinda " (*Citrulls vulgaris*, var. *fistulosus*), pulses such as " mothe " (*Phaseolus aconitifolius*), ' mung ' (*P. mungo*), 'mash (*P radiatus*), " desi-sem " (*Canavalia ensiformis*), " ghiatori " (*Luffa ægyptica*) and sweet-potato (*Ipomæa batatas*) They remain active upto December when their numbers gradually begin to decline until by end of February they disappear altogether

Tetranychus mori sp nov.

(Fig. 2, 2 *a*, 2 *b*, 2 *c* 2 *d*, 2 *e*)

Female :

Length (without palps) on slide : 0·38–0·47 mm.

Breadth on slide : 0·18–0·23 mm. (broadest on either side of dorsal furrow)

Segmentation of body distinct, dorsum with four transverse lines second and fourth being visible along the margins only (Fig. 2). *Coloration* : greyish-green to yellow-green with a few black spots scattered over the dorsum in

older specimens. *Chætotaxy* (Fig. 2) dorsum with fourteen pairs, a pair on either side of apex minute, third and fourth pairs with dorsal furrow in between and sub-frontal bristle arising almost from the line joining the anterior margins of eyes.

Eyes deep red, regular and single-corneated, *Mandibular plate* unnotched anteriorly, with length to breadth ratio 2 : 1 *Collar trachæ* (Fig 2) a straight tube with the distal end formed into an oblong undivided cell *Palp* (Fig. 2 *b*) . fifth segment with two hairs arising below claw, but one close to base small, '*thumb*' with a hair and three bristles, two between cone and dorsal finger, *cone* finger-like 2 5 μ broad, very long and equal in length to that of shorter sensillæ, each being 6·5 μ, other sensillæ near cone 7·5 μ in length, dorsal finger only 1 5 μ and shaped like a thorn Claw moderately developed and strongly curved *Legs* · first pair longest and always shorter than body, fourth pair shorter than first but longer than second and third which are equal (Statement V)

STATEMENT V

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·05	0·03	0 08	0 01	0·05	0·07
II & III	0·04	0 025	0 06	0 03	0 04	0·06
IV	0 04	0·03	0·06	0 01	0·05	0·07

Claws of legs similar to those of *T cucurbitæ* (Fig 1 *f*)

Male :

Length (without palps) on slide 0 29–0 32 mm

Breadth on slide . 0·12–0 18 mm (broadest along the posterior margin of cephalothorax)

Segmentation similar to that of female, all the four dorsal lines distinct. *Coloration* and *Dorsal chætotaxy* also similar to the female

Eyes, *mandibular plate* and *collar trachæ* like those of female. *Palps* (Fig. 2 *a*) . fifth segment with three hairs, two shorter and arising near base of claw, *thumb* with a hair and a bristle, between claw and dorsal finger, *cone* very much reduced and equal in length to dorsal finger, which is 2 5 μ long ; two sensillæ between them long being 6·0 μ and 4·0 μ, one nearer

cone longer. Claw moderately developed but weakly curved. *Legs* : First pair longest, but always shorter than body, fourth pair shorter than first but longer than second and third which are equal (Statement VI).

STATEMENT VI

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·04	0·02	0·06	0·03	0·04	0·06
II & III	0·03	0·015	0·055	0·03	0·035	0·05
IV	0·04	0 015	0·06	0·03	0·045	0·06

Claws of the legs similar to those of *T cucurbitæ*, except the first, which is bifurcated (Fig 2 *e*)

Penis (Fig. 2 *c*) 13·0 μ long. strongly chitinized, more or less triangular with apex curved

Biological notes —Tetranychus mori infests the lower surface of mulberry (*Morus alba*) leaves and is usually found in the depression between the mid-rib and the lamina It is a poor web-spinner and usually lays its eggs along the veins

It remains active during May–October In early November, when the plant sheds its leaves, the gravid females migrate to the buds where they overwinter. They become active towards the end of March when they lay eggs

Tetranychus fici, Hirst

(Figs 3, 3 *a*, 3 *b*, 3 *c*, 3 *d*, 3 *e*)

This species was described by Hirst (1926)[5] from the collection sent by Cherian from Coimbatore He figures only the penis and palpus tarsus of the male It is redescribed here in greater detail

Female :

Length (without palps) on slide. 0·37–0·48 mm.

Breadth on slide : 0·20–0·25 mm (broadest on either side of the dorsal furrow).

[5] Hirst, S., *Pro. Zool. Soc. London*, 1926, pp. 825–41.

Segmentation of body distinct, dorsum with three transverse lines (Fig. 3). *Coloration*: greenish yellow to yellow with a few grey blotches scattered all over the dorsum *Chætotaxy* (Fig. 3). dorsum with fourteen pairs of hairs, a pair on either side of the apex of abdomen minute and without an annular base, third and fourth pairs with the dorsal furrow in between and sub-frontal bristles arising almost from the line joining the anterior margins of the eyes

Eyes, deep red, circular, single-corneated, *mandibular plate* with anterior margin without notch, length to breadth ratio 2 1, *Collar trachæ* (Fig 3 *d*) like a bow, with the distal cell undivided and club-shaped *Palps* (Fig. 3 *b*) fifth segment with four bristles three located near the base of the claw, '*thumb*' with a hair, cone stout 5·0 μ in length and shorter than the rod-like sensilla near it which is 7·0 μ, dorsal finger and other two sensillæ 4 5 μ in length. Claw of medium size *Legs* first pair longest but shorter than body; fourth pair shorter than first but longer than second and third pairs which are equal (Statement VII).

STATEMENT VII

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·05	0·025	0·09	0·04	0·05	0·08
II & III	0 03	0·02	0·07	0·035	0·045	0·06
IV	0·04	0·025	0·09	0 04	0·05	0 08

Claws similar to these of *T cucurbitæ* (Fig 1 *f*).

Male:

Length (without palps) on slide 0 33–0·37 mm

Breadth on slide· 0·165–0·175 mm (broadest along the posterior margin of the cephalothorax).

Segmentation and chætotaxy similar to female *Coloration*· yellow to brownish yellow with brown and black spots scattered all over dorsum.

Eyes, *mandibular plate* and *collar trachea* like those of female' *Palps* (Fig. 3 *a*): fifth segment with four hairs, two near base of claw, '*thumb*' with one hair inserted on inner side, cone absent, three sensillæ present being

8·0 μ 7·0 μ, and 6 0 μ long, and shortest of sensillæ equal in length to dorsal finger Claws moderately developed but broad at base *Legs*: First pair longest but generally equal to or longer than body, fourth shorter than first but longer than second and third which are equal (Statement VIII).

STATEMENT VIII

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0 04	0 03	0·10	0 05	0·06	0·07
II & III	0 03	0·02	0·06	0 04	0·05	0·06
IV	0 035	0·025	0 06	0·045	0·055	0·06

Claws (Fig 3 *e*) similar to *T cucurbitæ* the anterior claws with a whorl of teeth and rest of the claws broken into six long hairs (Fig 1 *f*) Tennent hairs four

Penis (Fig 3 *c*) 24 0 μ long, nearly sickle-shaped, the outer curved portion on the same side as the shaft and the basal curve

Biological Notes —Tetranychus fici infests the lower surface of leaves and unripe fruits of figs (*Ficus carica*) only The attacked leaf develops white spots as a result of its feeding and in severe cases of infestation it gets slightly wrinkled, becomes yellowish brown and ultimately dries up and drops off the plant

It remains active during May–October In November, when the leaves start drying up due to cold, the gravid females migrate to the branches and lodge themselves on the terminal buds where they spend the winter. The buds open in the end of February or early March, when it infests them and thus starts the attack

KEY TO SPECIES OF GENUS PARATETRANYCHUS

Penis triangular with distal end curved (Fig 4 *c*) .. .. .. *Paratetranychus indicus*, Hirst

Penis lanceolate with distal end slightly bent (Fig. 5 *c*) .. .. *Paratetranychus mangiferus*, sp. nov.

Paratetranychus indicus, Hirst

(Figs 4, 4 *a*, 4 *b*, 4 *c*, 4 *d*, 4 *e*, 4 *f*, 4 *g*, 4 *h*)

This species was described by Hirst (1923)* from collection sent from Coimbatore. His figures show variation in the size and number of the finger-like projections on the thumb of male and female palps We have found the number of these structure identical in both sexes This species is redescribed here in the light of the additional diagnostic characters

Female :

Length (without palps) on slide · 0·36–0·53 mm

Breadth on slide 0 27–0 34 mm. (broadest on either side of dorsal furrow)

Segmentation (Fig 4) indistinct, only dorsal furrow being evident. *Coloration*: greyish green with black blotches scattered over the dorsum, colour becomes deeper and blotches increase with age Cephalothorax and appendages slightly lighter in shade *Chætotaxy* (Fig 4) dorsum with thirteen pairs of hairs, a pair on either side of the apex of abdomen minute, third and fourth pair with the dorsal furrow in between and sub-frontal bristles arising slightly ahead of the line joining the anterior margins of the eyes

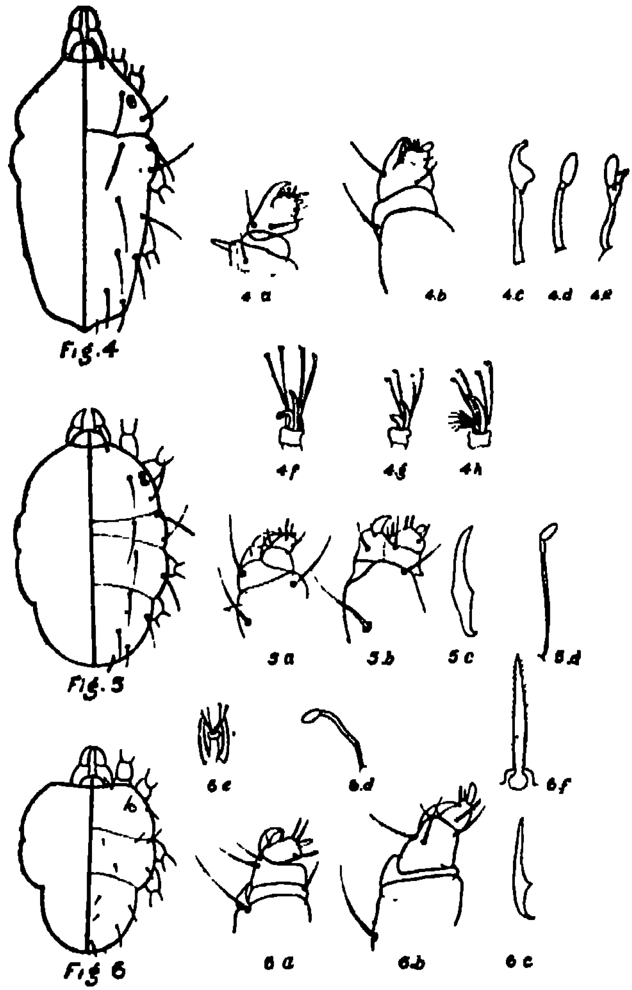
Eyes deep red, irregular and single-corneated *Mandibular plate* without the anterior notch, length to breadth ratio 30 18 *Collar trachea* generally ending in a single cell (Fig 4 *d*) but some may, however, end in two cells lying side by side (Fig 4 *e*) *Palps* (Fig 4 *b*) . Fifth segment with two long hairs , '*thumb*' with a hair and two bristles located between claw and dorsal finger Cone 5·0 μ long and 4·5 μ broad and equal in length to dorsal finger, sensilla near the cone 8 0 μ long more or less spine-like, and arising from a tubercle , other sensilla 6 0 μ long Claw small and weakly curved *Legs* · First pair longest, fourth shorter than first but longer than second and third, which are equal (Statement IX)

STATEMENT IX

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·06	0·04	0·11	0·05	0·06	0·10
II & III	0·04	0·03	0·09	0 045	0·06	0·09
IV	0·04	0·04	0·10	0·045	0·06	0·10

* Hirst, S., *Pro. Zool. Soc. London*, 1923, pp. 971–1000.



Paraletranychus indicus Hirst.

FIG. 4.—Showing shape and arrangement of hairs on dorsum of female. 4 *a*.—palp of male. 4 *b*.—palp of female. 4 *c*.—penis. 4 *d*. and 4 *e*.—collar trachea. 4 *f*.—claws of first leg of male. 4 *g*.—claw of second leg of male. 4 *h*.—claw of fourth leg of male.

Paratetranychus mangiferus sp. nov.

FIG. 5.—Showing shape and arrangement of hairs on dorsum of female. 5 *a*.—palp of male. 5 *b*.—palp of female. 5 *c*.—penis. 5 *d*.—collar trachea.

Anychus ricini sp. nov.

FIG. 6.—Showing shape and arrangement of hairs on dorsum of female. 6 *a*.—palp of male. 6 *b*.—palp of female. 6 *c*.—penis. 6 *d*.—collar trachea. 6 *e*.—terminal portion of legs. 6 *f*.—dorsal serrate hair.

Claws of all legs similar and equal in length but slightly shorter than those of the posterior legs of male, protuberances near their bases bear six bristles (Fig. 4 *h*).

Male :

Length (without palps) on slide · 0·33–0·38 mm

Breadth on slide : 0·20–0·22 mm. (broadest along the posterior margin of the cephalothorax).

Segmentation and dorsal chætotaxy similar to female (Fig. 4). *Coloration:* greyish green freshly emerged specimens without specks a few of which may be present in older specimens.

Eyes, mandibular plate, collar trachea like those of female *Palps* (Fig 4 *a*), number and location of bristles and finger-like projections on fifth segment and '*thumb*' identical with those of female. Cone 2·5 μ broad throughout and equal in length to dorsal finger and shorter sensilla, each being 4·0 μ : other sensilla 6·0 μ long, and more or less spine-shaped Claw moderately developed. *Legs* : First pair longest equal to or shorter than body, fourth pair shorter than first and second, which are equal (Statement X).

STATEMENT X

Showing the Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·05	0·04	0·00	0·05	0·055	0·07
II & III	0·04	0·03	0·07	0·04	0·045	0·06
IV	0·04	0·035	0·08	0·04	0·06	0·07

Dorsal portion of claws formed into a curved spur, which is longer in third and fourth pairs ; ventral portion in first and second pairs formed into hooks ending in teeth (Figs 4 *f* and 4 *g*), but in third and fourth pairs it remains a protuberance like structure which bears six bristles (Fig. 4 *h*).

Penis (Fig 4 *c*) 28·0 μ long, more or less triangular with the apex strongly curved.

Biological Notes.—Paratetranychus indicus does serious damage to sugarcane (*Saccharum officinarum*) in May–July, and sorghum (*Sorghum vulgare*) in May–July and September–October. It is present throughout the year on 'Buru' (*Sorghum halepense*) and in early May it is carried by wind

to other host-plants. The attacked portion of the leaves shows characteristic red coloration, in severe cases of infestation these red areas coalesce together, the whole leaf presenting red appearance. Such leaves dry and fall off. This kind of damage continues upto June–July when monsoon rains kill it and stimulate plant growth. The eggs, which remain unaffected, however start future infestation

Paratetranychus mangiferus sp. nov.

(Figs. 5, 5 *a*, 5 *b*, 5 *c*, 5 *d*)

Female :

Length (without palps) on slide : 0·35–0·47 mm.

Breadth on slide : 0·28–0·35 mm. (broadest along the anterior margin of abdomen.

Segmentation distinct with three transverse lines on the dorsum (Fig. 5). *Coloration* : dark red along the sides, area anterior to the dorsal furrow and along the mid-dorsal line pale red, the latter becoming darker with age, appendages, also paler in shade. *Chætotaxy* (Fig 5) dorsum : with thirteen pairs of hairs, a pair on either side of apex minute and without an annular base, third and fourth pairs with dorsal furrow in between and sub-frontal bristles arising behind line joining anterior margins of eyes

Eyes, red double-corneated (Fig 5), the anterior one darker in shade and circular in outline *Mandibular plate* without anterior notch, length to breadth ratio 20 · 13. *Collar trachea* straight, ending in single bulbiform cell (Fig. 5 *d*). *Palps* (Fig. 5 *b*) · Fifth segment with three hairs ; '*thumb*' with a hair and a bristle ; cone 3·25 μ long and slightly longer than broad, dorsal finger club-shaped and equal in length to cone, two sensillæ of shape as dorsal finger and 6·0 μ and 5·0 μ in length Claw moderately developed, broad at base. *Legs* : First pair longest and always shorter than body, fourth pair shorter than first but longer than second and third which are equal (Statement XI)

STATEMENT XI

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·04	0·02	0·09	0·045	0·055	0·09
II & III	0·03	0·02	0·07	0·035	0·04	0·07
IV	0·03	0·02	0·08	0·04	0·06	0·09

Claws about half the length of the tennent hairs and similar to those of *P. indicus* (Fig. 4 *h*).

Male:

Length (without palps) on slide: 0·30–0·34 mm

Breadth on slide: 0·20-0·22 mm (broadest along the posterior margin of cephalothorax).

Segmentation and dorsal chætotaxy similar to female (Fig 5). *Coloration*: dark red with appendages and cephalothorax paler in shade.

Eyes, mandibular plate and *collar trachea* like those of female. *Palps* (Fig. 5 *a*); Fifth segment without any hair, 'thumb' with a hair and two bristles; cone very much reduced being only 1·5 μ in length, sensilla near cone 5·0 μ and equal in length to dorsal finger Other sensilla 7·0 μ long. Claw moderately developed but weakly curved *Legs*: First pair longest but shorter than body, fourth pair shorter than first but longer than second and third, which are equal (Statement XII)

STATEMENT XII

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·04	0·02	0·08	0·04	0·05	0·06
II & III	0·03	0·02	0·05	0·03	0·04	0·05
IV	0·03	0·02	0·06	0·03	0·045	0·06

Claws similar in structure to *P. indicus* (Fig. 4 *f* and 4 *g*) but shorter in length. Tennent hairs also comparatively shorter

Penis (Fig 5 *c*) 31·0 μ long lanceolate and curved on same side as base.

Biological Notes.—Paratetranychus mangiferus infests upper surface of Mango (*Mangifera indica*), 'Jaman' (*Eugenia Jambolana*) and grape-vine (*Vitis vinifera*) leaves; it breeds throughout the year on the leaves of the first two named plants. The web spun is quite profuse and the attacked leaves show extensive mottling. Those on grape-vine leaves winter over on the buds as this plant is denuded of its leaves in December The buds burst in March and the mites are available on the leaves by the end of April. There may be thirty-nine mites in an area of six square inches without producing any ill-effects on the plant. Monsoon rains kill all of its stages excepting the eggs, which produce future progeny.

GENUS ANYCHUS

There is only one species included in this genus, *viz.*, *Anychus ricini*.

Anychus ricini sp nov.

(Figs. 6, 6 *a*, 6 *b*, 6 *c*, 6 *d*, 6 *e* and 6 *f*)

Female :

Length (without palps) on slide · 0·38–0·54 mm.

Breadth on slide: 0·33–0·44 mm. (broadest on either side of dorsal furrow).

Segmentation distinct, two transverse lines being visible on dorsum (Fig 6). *Coloration* : greenish brown, greenish tinge increasing with age and before death they become more or less black : appendages and cephalothorax light brown *Chætotaxy* (Fig 6) ; dorsum with thirteen pairs of stout serrate hairs arising from prominent tubercles (Fig. 6*f*), 2 more pairs of hairs on either side of the apex of abdomen without tubercular base and hairs on them Hairs along the mid-dorsal line rather stump-like as compared to those arising along the periphery , third and fourth pair with dorsal furrow in between and sub-frontal bristles arising posterior to the line joining the anterior margins of the eyes.

Eyes red, circular in outline and single-corneated , *Mandibular plate* without anterior notch, length to breadth ratio 44 : 33 *Collar trachea* (Fig. 6*d*) more or less straight with a single undivided distal cell. *Palps* (Fig 6*b*) 5th segment with 5 hairs, 2 located near base of claw short, others, very long , '*thumb*' with a single serrate hair on inner side below cone, cone finger-like 8·0 μ long and 2·8 μ broad, dorsal finger vestigeal, sensillæ shorter than cone and are 7·0 and 5·0 μ in length. *Legs:* shorter as compared to male, first longest, fourth shorter than first but longer than second and third which are unequal (Statement XIII).

STATEMENT XIII

Showing Length (in mm.) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·04	0·03	0·14	0·07	0·08	0·09
II	0·035	0·025	0·12	0·065	0·07	0·08
III	0·035	0·025	0·11	0·06	0·07	0·08
IV	0·035	0·03	0·11	0·06	0·08	0·09

Claws of legs absent, terminal portion with four tennent hairs arranged in two groups of two each (Fig. 6 *e*).

Male :

Length (without palps) on slide. 0·30–0·40 mm.

Breadth on slide: 0·24–0·28 mm. (broadest along anterior margin of dorsal furrow).

Segmentation and chætotaxy like those of female (Fig. 6). *Coloration* : generally light brown, the older specimens may, however, develop greenish tinge. Cephalothorax and appendages comparatively lighter.

Eyes, mandibular plate and *collar trachea* like those of female *Palps* (Fig 6 *a*) : fifth segment with two long hairs arising near base of claw; '*thumb*' with a bristle and a hair, located on inner side below cone, serrate. Cone finger-shaped, 6 0 μ long and 2 0 μ broad, dorsal finger 4·0 μ and two sensillæ more than twice the length of dorsal finger being 9 0 μ Claw well-developed and strongly curved. *Legs* : very long, first pair longest, and second shorter than first but longer than third and fourth, which are unequal (Statement XIV). Legs first, second and fourth longer than body.

STATEMENT XIV

Showing Length (in mm) of Leg Segments

Leg	Coxa	Trochanter	Femur	Patella	Tibia	Tarsus (without tennent hairs)
I	0·045	0·045	0·15	0·08	0·10	0·09
II	0·04	0·04	0·15	0·07	0·08	0·08
III	0·04	0·04	0·10	0·05	0·08	0·08
IV	0·04	0·04	0·10	0·05	0·09	0·09

Terminal portion of legs similar to that of female (Fig 6 *e*).

Penis (Fig. 6 *c*) : 34·0 μ long, strongly chitinized distal portion weakly curved on the same side as shaft and basal portion

Biological Notes —Anychus ricini infests upper surface of the leaves of castor (*Ricinis communis*), almond (*Prunus amygdalus*), 'ammaltas' (*Cassia fistula*) "ber" (*Zigiphus jujuba*) and *citrus*, its attack being very severe on first three named plants. Sour lime and oranges are more seriously infested as compared to other varieties of citrus.

It is active throughout summer but during the monsoon rains all stages except eggs which produce future progeny are killed. During winter mostly its gravid females and eggs are available on the leaves of the host-plants.

Acknowledgment

We are grateful to Khan Bahadur M. Afzal Hussain, Entomologist to Government, Punjab, Lyallpur (now Vice-Chancellor of the Punjab University, Lahore), for suggesting the problem and for helpful encouragement during its progress.

ON THE ANATOMY OF *LYCOPODIOPSIS DERBYI* RENAULT WITH REMARKS ON THE SOUTHERN PALÆOZOIC LYCOPODS*

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CONTENTS

1. *Introduction*

Lycopodiopsis Derbyi is one of the few interesting silicified plants known from Brazil, the other especially notable types being *Tietia singularis* Solms. and *Psaronius brasiliensis* Unger. All these fossils, and particularly *Tietia* and *Lycopodiopsis*, are in need of further investigation.

The genus *Lycopodiopsis* was founded by Renault in 1890 (Renault, 1890[1]; 809–11; 1890,[2] 109) on two fragments sent to him by Dr. Orville A. Derby of Rio de Janerio. The fossils were discovered at Piracicaba, São Paulo, Brazil (Zeiller, 1895, 604), in rocks then classed as "Permo-Carboniferous" in age. The fact that *Lycopodiopsis* occurs associated with typical members of the Glossopteris flora (White, 1908, 363) invests this plant with special interest, for the genus has sometimes been indentified with *Lepidodendron*, one of the most characteristic members of the so-called "northern"

* This paper formed part of the thesis on which Mr. H. S. Rao was recently declared eligible to receive the D.Sc. degree of the University of Lucknow.

coal measure flora. This genus, like some other "northern" types, is therefore supposed to have migrated southwards to the Brazilian part of the Gondwana continent. A similar association (in this case of *Sigillaria* and *Glossopteris*), was recorded by Professor Sir A. C. Seward from South Africa a little over forty years ago (Seward, 1897), and since then other instances have been observed where "northern" forms occur in Gondwana land (see, especially, Walton, 1929).

The distribution of *Lycopodiopsis Derbyi* is much wider than is generally supposed. Oliveira (1937, No. 10, p. 13) mentions five localities in South America : Tubarão, Iraty, Piracicaba, Estrada Nova and Bofete in São Paulo.

Oliveria regards the age of the beds in which *Lycopodiopsis* is found, as definitely Permian and not "Permo-Carboniferous". He, however, does not discuss the age (*loc. cit.*, 12). Roxo (1938, 15 ; legend to Fig. 15) also refers to the age as "Permiano".

The main character distinguishing *Lycopodiopsis* form *Lepidodendron* is the discontinuous nature of the xylem cylinder. Renault (1890[2]) gave a number of figures to show this feature, but as this material appears to have been poorly preserved the figures are not quite clear.

Later, Zeiller (1898, 245) re-examined these specimens and came to the conclusion that they really belonged to a *Lepidodendron*, the stele being according to him a continuous cylinder, very like that in *L. Harcourtii*, and the exit of the leaf-trace as in *L. selaginoides*. He says the apparently parenchymatous rays between the bundles are really composed of badly preserved woody elements, the walls of which appear thin owing to alterations which have taken place during fossilisation. Zieller further says that partial decortication explains the difference between the external features of *Lycopodiopsis* and *Lepidodendron*. This is quite evident from a comparison of our Pl. IX, Figs. 1 and 2. He suggests that *Lycopodiopsis* may be the petrified form of *Lepidodendron Pedroanum*, a species which is known only as impressions, and possesses acicular leaves.

E. A. Newell Arber (1905, 159–62) described the species under the name *Lepidodendron Derbyi* (Renault). His account is largely a quotation from Renault (1890[1]). The material described by Arber was in the Museum d'Historie Naturelle, Paris. It is not clear from his account whether this material is different from Renault's original specimens. He merely says that *Lepidodendron Derbyi* is known only from Brazil.

In 1908 White described *Lycopodiopsis Derbyi* (White, 1908, 436, Pl. V, Figs. 11, 11*a*) from another locality in Brazil : Bofete, São Paulo, horizon

about 155 metres above the Iraty Black Shale. He upholds Renault's genus and records it as one of the characteristic genera of the lower Gondwana flora. He compares it closely with Prof. Seward's South African species *Bothrodendron Leslii* (Seward, 1910, II, 258) which White regards as a *Lycopodiopsis*.

Hirmer (1927, 317) opines that *Lycopodiopsis* should be kept as a distinct genus.

Professor Seward does not mention *Lycopodiopsis* in his "Plant Life through the Ages" (1933) probably because of Zeiller's view that it is a *Lepidodendron*, and lack of any confirmatory description of the internal anatomy.

Steinmann (1924) upholds Renault's genus, affirming that the stele is not continuous, and says that there seems to be no perichnos. He, however, figures only the surface features, though he says his material is "ausgezeichnet erhalten".

Oliveira (1937) has recently published a useful summary of the present position of Brazilian Palæobotany where he mentions *Lycopodiopsis Derbyi*. He treats of the subject as a geologist, that is, age-wise, after giving a historical sketch. He gives a full bibliography, but no figures.

The latest mention of *Lycopodiopsis*, so far as I know, is by Roxo (1938) who gives a summary of our knowledge of Brazilian fossil plants, treating of them botanically and giving many illustrations. He includes a figure (his Fig. 15 is here reproduced as Pl. IX, Fig. 1), showing the external features of *Lycopodiopsis* based on a specimen in the Palæontological Museum of the Geological Survey of Brazil.

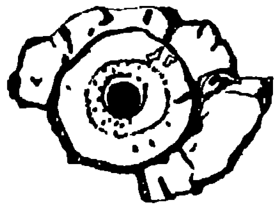
Lycopodiopsis being an exclusively southern genus, and regarded by White as a characteristic member of the *Glossopteris* flora, a better knowledge of its anatomy is desirable. The following brief account tends to confirm Renault's idea that the genus is distinct from *Lepidodendron*. At the same time a few details of the anatomy have been added so far as the preservation of the material allowed.

2. *Description*

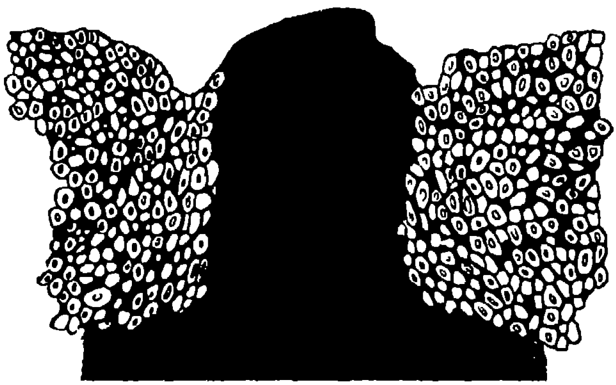
(*i*) *The Material.*—Several specimens of *Lycopodiopsis* are known. The present description and figures are based only on two of these, which were borrowed for reinvestigation by Professor Sahni from Museums in Europe, but which he kindly passed on to me as he could not find the time to describe them himself.

(*a*) The first specimen is a fragment from a silicified stem preserved at the École des Mines, Paris. It was borrowed by Professor B. Sahni

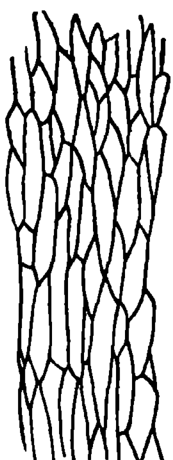
during his visit to Paris in 1930, through the courtesy of M. Painvain, keeper of the palæontological collections of the École. The locality is given as Tatuhy, State of São Paulo, Brazil. This specimen is shown in Pl. IX, Figs. 2, 3 and 3*a*; Pl. X, Figs. 6–8; and Text-Figs. 2–4 and 6. Pl. IX, Figs. 2, 3



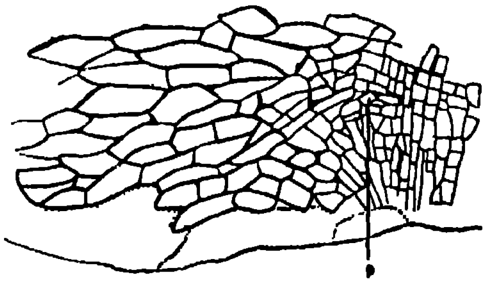
TEXT-FIG 1 Sketch of the Bonn specimen Sections were made from this slice × 1.



TEXT-FIG. 2. The Paris specimen Transverse section of a leaf-cushion Note the thickened cells of the same × 36



TEXT-FIG. 3. The Paris specimen : Longitudinal section of a leaf-cushion, showing the elongated cells. × 36.



TEXT-FIG. 4. The Paris specimen Longitudinal section of leaf-cushion showing the abscisslayer "a" The line below marks the surface Between it and the well-preserved tissue the preservation is imperfect × 36

and 3*a* show well the armour of persistent leaf cushions, but owing to partial decortication the original external surface is no longer visible (see Fig. 1).

(*b*) The second specimen here described was the orignal of Steinmann's description. This specimen is particularly valuable as it has yielded indubitable proof of the discontinuous nature of the stele, illustrated in Text-Fig. 5. Steinmann, as already stated, had only figured the external features. The material was obtained on loan by Professor Sahni during his visit to Europe in the summer of 1935, from Professor N. Tilmann of the University of Bonn.

A comparison of the Paris and Bonn specimens—the present account deals with these two—leaves no doubt that they belong to one species. Although differing in size and also somewhat in their external appearance, owing to varying degrees of decortication, they agree in their anatomical characters.

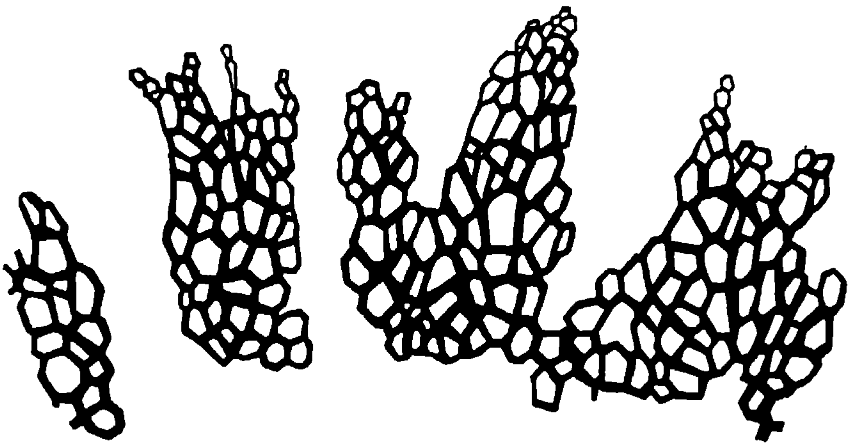
(*c*) During a recent tour in Europe (1935) Professor Sahni examined a third specimen in the British Museum (No. V, 13430) This is labelled "*Lepidodendron Derbyi* (Renault)", and is stated to have come from Piracicaba, S. Paulo, Brazil, the original locality from which Derby had collected the fragments he sent to Renault. The age is given as "Permo-Carboniferous".

I have not been able to examine the British Museum specimen, but have had the advantage of consulting some notes and sketches (dated London, June 3, 1935) made by Professor Sahni. The specimen is a fragment of a flattened stem including a length of about an inch and a half. The external surface shows a number of small more or less rhomboid areas corresponding to the leaf cushions, each showing a little scar near the upper (distal) angle of the rhomboid. These features are well seen in a cast, also preserved

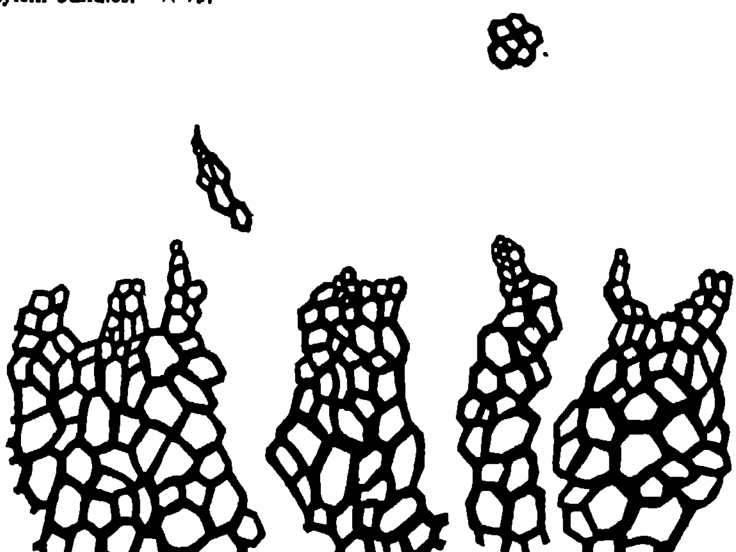
with the silicified original. A thin selection cut near one end (V, 13430*a*) shows the armour of leaf cushions; in spite of the fact that the stem is considerably crushed the eccentrically placed stele is perfectly circular in section. Professor Sahni states that the diameter of the stele is somewhat larger than in the Paris fragment he had borrowed in March 1930. This specimen is also no doubt somewhat decorticated, but evidently much less so than in the Paris one, of which the surface shows no sign of rhomboid areas. Apart from this difference there is nothing to suggest that the two specimens are not specifically identical.

(*ii*) *External features.*—In Pl. IX, Fig. 1 is reproduced a photograph of an impression of *Lycopodiopsis Derbyi* published by Roxo (1938, Fig. 15; the figure seems to be upside down, hence in my reproduction of it, it has been turned round through 180°). The specimen came from the Permian of the State of São Paulo. The external features of Steinmann's specimen have already been well described by him. The Paris specimen, shown in Pl. IX, Fig. 2, is a nearly cylindrical piece of stem 24 mm. long, with a diameter of about 20 mm. The surface shows spirally arranged leaf scars, which being partly decorticated, are represented by shallow depressions with an oval knob placed within them. The transverse section (Pl. IX, Figs. 3, 3*a*) shows about 20 leaf cushions at the periphery. The specimen is filled with agate-like concretions in the badly preserved cortical region. This feature is also seen in Steinmann's specimen.

(*iii*) *Internal anatomy.*—In both the specimens the pith is about 2 mm. in diameter. The tissues are not well preserved. The London stem, Professor Sahni informs me, has a slightly larger pith. It consists of polygonal, isodiametric cells which are slightly larger at the centre than at the periphery. These features are better seen in the Bonn speimen (Pl. X, Fig. 5). The pith is surrounded by a ring of exarch bundles often united internally in twos or threes in the form of a U, V or W (Pl. X, Figs. 5–6; Text-Figs. 5 and 6). The narrowest elements are at the outer extremity of the bundles. The tissue between the bundles is not well preserved, but enough is seen to make it certain that it consists of narrow and thin-walled cells of the ground tissue, and not of disorganised xylem elements as Zieller had thought. From the "bays" of the U, V or W shaped bundles arise the leaf-traces (Pl. X, Figs. 5–6). They consist of a few elements, the whole group being usually markedly flattened in the radial direction, with the narrowest tracheids in an exarch position. Just outside the xylem ring is a parenchymatous zone, containing a number of dark patches which appear like secretory sacs. The secretory sacs in the closely related genus *Lepidodendron* are said to function as a phlœm (Seward, 1902, 41).



TEXT-FIG. 5. The Bonn specimen Transverse section showing the gaps between the exarch xylem bundles. × 75.



TEXT-FIG 6. The Paris specimen Transverse section showing the discontinuous exarch xylem bundles and the two leaf-traces (see near ends of black lines, Pl X, Fig. 6). One leaf-trace is flattened in the radial direction × 75

The stele is on the whole better preserved in Steinmann's specimen than in the Paris one. The cortex is not preserved in either of them and shows only very faintly the course of the leaf-traces. The spiral or annular tracheids of the latter are better seen where the leaf-traces pass though the leaf-cushions (Pl. IX, Fig. 4).

The tissue of the leaf-cushions (Pl. X, Figs. 7–8; Text-Figs. 2–4) is well preserved in the Paris specimen. The thick-walled cells are isodiametric in transverse section (Pl. X, Fig. 8: Text-Fig. 2), but elongated vertically (Pl. X, Fig. 7; Text-Figs. 3–4). In the leaf cushions, as seen in a transverse section of the stem, the tracheids of the outgoing leaf-traces show spiral or annular thickenings. The longitudinal section of the leaf-cushion shows what may be an absciss layer consisting of narrow rectangular cells (Pl. X, Fig. 7 see *a* ; Text-Fig. 4 *a*). No trace of a parichnos can be made out in either of the specimens, but it may be due to the imperfect preservation. The two dots in the upper half of the leaf-cushion seen in Roxo's photograph (Pl. IX, Fig. 1) may mark the parichnos. The rather larger round hump seen in some of the cushions in the upper half may be the leaf-trace scar.

The exarch siphonostele, the leaf-traces arising from the " bays " and composed of a few elements sometimes flattened in the radial direction, with the narrowest tracheids in an exarch position, the spirally arranged leaf-cushions clearly rhomboid in the London specimen (and in Roxo's specimen), and the secretory sacs in the region of the phlœm, are features indicating a general Lepidodendroid affinity. But the most important difference from *Lepidodendron* is the discontinuity of the xylem, which Steinmann's specimen shows to be a fact beyond question (Pl. X, Fig. 5). Zeiller's explanation that it is due to imperfect preservation does not hold good. The institution of a separate genus *Lycopodiopsis* therefore seems justified.

Affinities: Roxo (1938, 15) refers *Lycopodiopsis Derbyi* to the Lycopodiales, and the genera *Lepidodendron*, *Sigillaria*, etc., to the Lepidodendrales. If the Lepidodendrales are at all to be separated as a phylum from the Lycopodiales then *Lycopodiopsis* should without hesitation be assigned to the former group, not the Lycopodiales. Both in the external features and in the stelar anatomy and mode of leaf-trace origin the resemblance to *Lepidodendron* is far stronger than the resemblance to *Lycopodium* or *Selaginella*.

3. *General Remarks on the Southern Palæozoic Lycopods*

This re-examination of *Lycopodiospis Derbyi*, a member of the *Glossopteris* flora of South America, suggests a consideration of the southern *Permo*-Carboniferous lycopods in general.

The existence of late Palæozic lycopods in Gondwana land in association with typical members of the *Glossopteris* flora raises the question, *firstly*, of the relation between the floras of the northern and southern hemispheres at the time and, *secondly*, the relation between the pre-Gondwana and Gondwana floras.

The great southern glacial climate at the beginning of the so-called *Permo-*Carboniferous time (Upper Carboniferous to, at the most, late Middle Permian) ushered in an essentially new flora. The Gondwana flora, when it arose, was very distinct from the contemporary flora of the northern hemisphere. But in addition to the new and typically southern plants that arose the Gondwana flora comprised also some elements of the so-called "northern" flora. It might well be asked whether these latter are immigrants from the north or descendants of a pre-Gondwana stock which at that time had a distribution in both the northern and southern hemisphere.

As early as 1897 Prof. Sir A. C. Seward had already discussed the problem raised by the presence of Lepidodendroid and Sigillarian plants in association with the *Glossopteris* flora in South America and South Africa (Seward, 1897, p. 336). The alteranatives, as stated by him, were: "(i) Was there a land connection between the continent of Gondwana land and the northern continental areas towards the close of the Palæozoic epoch? (ii) May we regard the Lepidodendroid and Sigillarian species of South America and South Africa as survivals from an older period which preceded the typical *Glossopteris* flora?"

The first question would meet with an excellent answer if Wegener's fascinating theory of continental drift be correct. According to this theory, all land on earth formed one single continental mass, the "Pangaea", before the Upper Carboniferous period (Wegener, 1924, 6). This might explain the occurrence of links between the northern and southern floras and the persistence of some of these links into a later period when the disruption of Pangaea has already gone on for some time.

With regard to the second question Arber's (1905, 31) view was that the southern lycopods are "probably only represented in the *Glossopteris* flora by migrations from the northern continent and were not indigenous to Gondwana land." This remark would apply to such forms as *Lepidophloios laricinus*, *Lepidodendron Sternbergii* and *Sigillaria Brardi*.

On the other hand there are a few forms which, it seems, occur both in the pre-Gondwana and in the Gondwana beds. Thus *Lepidodendron Pedroanum* occurs both in the Lower Carboniferous (? Upper Devonian) of Argentina and the Gondwana beds of Brazil and South Africa (see Tables). *L vereenigingense* found in South Africa has been closely compared with the pre-Gondwana forms, *L. Volkmannianum* and *L. nothum* from New South Wales. *Sigillaria australis* has been found in the Upper Carboniferous of Brazil, as well as in the Gondwana beds of the same country, associated with the *Glossopteris* flora. In the case of these plants it seems

equally likely (*a*) that they migrated to Gondwana land from the north, or (*b*) that they are descendants of a hardy stock that withstood the rigours of the southern Palæozoic Ice Age.

Lastly, there are a few lycopods with a purely southern distribution, these are *Bothrodendron Leslii* Sew. and *Sigillaria* (?) *muralis*.

A definite decision on these difficult questions cannot be given with our present data. But there is the fact of the wide distribution of the pre-Gondwana lycopods in both the hemispheres. There is also the important fact that a number of forms are common to both pre-Gondwana and Gondwana beds. Hence Professor Sahni (1926, p 241) inclined towards Prof. Seward's second suggestion (see above) and said, "I believe it is not unlikely that at least some of them are hardy survivors (and in part descendants) of the cosmopolitan life of pre-Gondwana times."

With a view to see how far it is possible to substantiate the latter view I have prepared a list of the Gondwana lycopods from the southern hemisphere (see Tables I, II). It would be an advantage if one could have examined the originals of all the specimens listed, or at least their illustrations. Even the latter has not always been possible. I have had to rely on the descriptions given by the various authors, and the views expressed by those who have had better opportunities than myself of assessing the affinities of species.

Table I lists the pre-Gondwana lycopods of the southern hemisphere. At least nine of these are comparable in greater or less degree (see Remarks column in the table) to northern forms. They are : (1) *Lepidodendron, Osborni* (2) *L. Veltheimianum*, (3) *L. rimosum*, (4) *L. comp dichotomum*, (5) Lepidodendroid stems from the Falkland Islands, (6) *Leptophlæum australe*, (7) *Protolepidodendron lineare*, (8) *P. Yalwalense* and (9) *Ulodendron minus*.

The exclusively southern lycopods are : (1) *Lepidodendron Clarkei*, (2) *L. obovatum*, (3) *L. Volkmannianum*, (4) *L. australe*, (5) *L. nothum*, (6) *L. Pedroanum*, (7) *Cyclostigma* and (8) *Haplostigma irregulare*. Thus the majority of the known southern pre-Gondwana lycopods had a cosmopolitan distribution. Those listed as exclusively southern were probably locally differentiated species confined to restricted regions.

Now, turning to the Gondwana lycopods (Table II) it is seen that only *Lepidophloios laricinus*, *Lepidodendron Sternbergii* and *Sigillaria Brardi*, are closely comparable to northern forms. All the others are typically southern.

These latter are : (1) *Lepidodendron Pedroanum*, (2) *L. vereenigingense*, (3) *Lycopodiopsis Derbyi*, (4) *Sigillaria australis* and (5) *Bothrodendron Leslii*. Of these it is probable that *Lepidodendron Pedroanum* and *Lycopodiopsis Derbyi* are one and the same. *Lepidodendron Pedroanum*. *L. vereengingense* and *Sigillaria australis* occur both in Gondwana and pre-Gondwana beds.

From the above analysis the great reduction in the number of lycopods, after the glacial conditions which ushered in the *Glossopteris* flora, becomes noticeable. Thus, Professor Sahni's tentative view that they are hardy survivors and in part descendants of the cosmopolitan life of pre-Gondwana times would gain distinct support. If Zeiller's conjecture is justified that *Lepidodendron Pedroanum* and *Lycopodiopsis Derbyi* are one and the same, then *Lycopodiopsis Derbyi* would be classed as one of these surviving links with the preglacial flora.

The idea that the great Southern Ice Age completely extinguished the original flora over the whole of the Gondwana continent was already very improbable (Sahni, 1938, pp. 10–18; 1939, pp 1–6) but it has been set at rest by the recent discovery of spores of plants within the matrix of a Gondwana tillite from Australia, and of a fairly well-developed flora of spores in the Salt Range of India, at horizons only 1½ feet and 4½ feet respectively, above the Talchir boulder bed (see Virkki, 1938, 1938*a* and 1939). There is thus no real objection, at least on climatic grounds, to the idea that some hardy members of the pre-glacial flora survived through the cold period and became associated with the newer elements composing the main part of the *Glossopteris* flora.

4. *Acknowledgments*

My thanks are due to Professor Sahni who not only suggested this work and entrusted the rare material to me, but also supplied me with valuable notes prepared during his European tours and gave me the benefit of his advice and criticism throughout the course of the investigation. I wish to offer thanks also to Professor Painvain of the École des Mines, Paris, and to Dr. Tilmann of Bonn, who lent the material to Professor Sahni. I am grateful to the authorities of Lucknow University for the award of a Research Fellowship during the tenure of which this investigation was made.

TABLE I

Pre-Gondwana Lycopods of the Southern Hemisphere

The species closely allied to or identical with Gondwana species in Table II are marked thus*
The species more or less comparable to northern hemisphere forms are marked thus †

Lycopod type	Locality	Age						Reference	REMARKS
		Devonian			Carboniferous				
		Lr.	Mid	Up	Lr.	Mid.	Up		
† *Lepidodendron Osborni*, Walkom	Volcanic stage of Kuttung series at Welshman's Creek, New South Wales.				×			Walkom, 1928	1 Close resemblance *L spetzbergense* Nath. from Lower Carboniferous of Spitzbergen. 2. Considerable resemblance to *L. rimosum* Sternb. (Zeiller), and 3 *L. Glincalum* (Kidston). 2 and 3 from Lower Coal Measures of Canonbie. 4. *Cf L. rimosum* var. *reticulosum* (White) from Lower Coal Measures of Missourie 5. *Cf L. rimosum* (Zalessky) from Carbonif of Donetz Poland and Valenciennes. 6. *Cf. L Kidstoni* (Nathorst) from Kulm of Spitzbergen.
L. Clarkei, Walkom	Yalwal, N.S.W.			×				Do.	
† *L. comp. dichotomum*, Stern.	Smith's Creek, Stroud, N.S W.				×			Feistmantel, 1890	
* *L. Volkmannianum* Stern.	Do.				×			Do.	Comparable to *L. vereenigingense*, a Gondwana form.
L. cf. *Volkmannianum*	Peru				×..	..?		Steinmann, 1910	

† *L. Veltheimianum*	1. Burindi Series, N.S.W.				×			Süssmilch and David, 1920.	*Cf.* 1. *L. dichotomum* of Europe. 2. *L. scutatum* of N. America.
	2. Star Series, Queensland.						×	Walkom, 1919	
	3. Drummond Series, Queensland.						×		
L. cf. *Veltheimianum*	Peru				×	?		Steinmann, 1910	
L. australe, Mc Coy.	1. Star Series, Queensland						×	Walton, 1926, Walkom, 1919; Feistmantel, 1890	Walton includes this sp. and *L. nothum* of Carruthers and Feistmantel under *Leptophlœum australe*, Mc Coy.
	2. Drummond Series Queensland.						×	Seward, 1907	
	3 Rockhampton Series, Queensland						×		
	4. Victoria.			? ×	×				
	5 Elandsdraai, near Orange River Station, S Africa				×				
	6 Dwyka Series, S Africa.						×		
	7 Murrumbidgean Series.	×		?					
* *L nothum*, Unger.	1 N S W.			×				Feistmantel, 1890	Comparable to *L. vereenigingense*, a Gondwana form.
	2. Queensland			×					
L cf. *nothum.*	Argentina						×	Szajnocha, 1891, Bodenbender, 1896	
* *L. Pedroanum* Carruthers	Retamito, Argentina			? × .	×			Szajnocha, 1891	*This is also represented in the Gondwana flora of Brazil and South Africa*
L. obovatum Stern	Peru				×			Berry, 1922	*Cf L. Rhodeanum.*
† *L. rimosum* Stern.	Do.				×			Berry, 1922	
Lepidodendron similar to *L. nothum* Unger.	San Juan, Argentina						? ×	Szajnocha, 1891	Age not definitely stated : said to be from the pre-*Glossopteris* flora.

TABLE I (*Contd.*)

Lycopod type	Locality	Age: Devonian Lr.	Devonian Mid.	Devonian Up	Carboniferous Lr.	Carboniferous Mid.	Carboniferous Up	Reference	REMARKS
Knorria state of *L. Veltheimanum*	Smith's Creek, Stroud, N S.W				×			Feistmantel, 1890	
† Lepidodendroid stems.	Port Purvis, Falkland Islands		×					Seward and Walton, 1923	Some resemblance with *Protolepidodendron* from Middle Devonian of Bohemia. Close resemblance with *Bothrodendron irregulare* Schw. from Witteberg beds of S. Africa. It is not easy to say whether these stems are referable to *Cyclostigma* (including some Upper Devonian Bothrodendra) or to the older Devonian *Arthrostigma* and *Protolepidodendron*.
Lepidodendroid stems	Half-way Cove, Falkland Islands		×					Halle, 1911	These recall *L. nothum* Ung. and *L. australe* Mc Coy.
Leptophloeum australe Mc Coy.	Queensland			× .	. .	. . .	. ×	Walton, 1926	*Cf Leptophloeum rhombicum* Dawson from Upper Devonian of Perry Basin, Maine, U.S.A. Walton includes *L. australe* Mc Coy. and *L. nothum* Unger. under this name.
Protolepidodendron lineare Walkom	Yalwal, Gold Field			? ×				Walkom, 1928	*Cf. P. primaevum* from Upper Devonian of New York. *Protolepidodendron* may be synonymous with *Archaeosigillaria*

P. Yalwalense Walkom	Do.			? ×				Do.	*Cf. Lepidodendron karakubense* from the Upper Devonian of New York.
Ulodendron minus, Lindley and Hutton	Volcanic stage of Kuttung series at Welshman's Creek, N.S W.				×			Walkom, 1928 and Süssmilch and David, 1920	1. Very similar to *Lepidodendron Veltheimianum* (figured by Kidston), from Carboniferous limestone of Midlothian. 2. *Cf U minus* and 3. *U. major* from Upper Culm of France 4. *Lepidodendron mirabile*, Culm of Spitzbergen.
Stigmaria ficoides Brongn.	In N.S W.: 1 Basal stage of Kuttung Series at Clarencetown 2 Volcanic stage of Kuttung Series, Welshman's Creek, Wallarobba 3. Rouchel.				× ×			Walkom, 1928	*Cf* specimens from the Upper Culm of Loire, France. It is possible that some specimens recorded as *Cyclostigma* may be this plant
Cyclostigma australe Feist.	1. Burindi Series, N S W. 2. Drummond Series, Queensland				×			Feistmantel, 1890; Walkom, 1919.	.
Cyclostigma sp.	1. Star Series 2. Rockhampton Series 3. Mt Wyatt beds, Queensland		× .	. . ×			× ×	Do	This is comparable to *Bothrodendron irregulare* Schwarz, from the Witteberg beds of South Africa. It is possible that some spp of *Stigmaria ficoides* from N.S W, may be this plant
Cyclostigma irregulare (Schwartz)	Witteberg Series, South Africa.		×					Do	

TABLE I (*Contd.*)

Lycopod type	Locality	Age						Reference	REMARKS
		Devonian			Carboniferous				
		Lr.	Mid	Up	Lr.	Mid	Up		
C. Leslii (Seward)	Bokkeveld Series, South Africa.	×						Do.	
Bothrodendron irregulare Schwartz	Witteberg Series of South Africa.			? ×				Seward, 1909, Seward and Walton, 1923.	*Cf. Cyclostigma* sp.
Haplostigma irregulare (Schwartz)	Bokkeveld Series		×					Seward, 1932	This is an intermediate type between the Psilophytales and Lycopodiales.
Lepidophyllum sp. .	Peru					×		Berry, 1922	
Lepidostrobus sp. .	Do.					×		Do	
Stigmaria sp. .	Do.					×		Do.	
Knorria sp. .	Do.					×		Do	
Sigillaria or *lepidodendron*	Do.				×			Seward, 1922	
* *Sigillaria australis* D. Wh. and spores and leaf of same	Rio Bonito beds of Coal Measures of Brazil						×	White, 1908	

TABLE* II

Gondwana Lycopods from the Southern Hemisphere.

The species closely allied to or identical with pre-Gondwana species in Table I are marked *.

Lycopod type	Locality	Occurrence	Reference	REMARKS
* *Lepidodendron Pedroanum* Carruthers	1. Serra Partida, Candiota 2. Arroyo dos Ratos 1 and 2 are in Rio Grande do Sul, Brazil. 3. Vereeniging, South Africa.	With *Glossopteris* flora.	Arber, 1905; Seward and Leslie, 1908; Bodenbender, 1896	Renault suggests that *Lycopodiopsis Derbyi* may perhaps be only a badly preserved stem of *L. Pedroanum*. *L. Pedroanum* also occurs at Retamito, San Juan, Argentina in pre-Gondwana beds.
L. Sternbergii Brongn (*L. Lycopodioides* Sternberg)	Argentina	Do	Arber, 1905, cites from Bodenbender, 1895	Occurs also in the Coal Measures of Shropshire (Seward, 1910, p. 97).
* *L. vereenigingense* Seward and Leslie	Vereeniging	Do.	Seward and Leslie, 1908	Comparable to: 1 *L. Volkmannianum* Stern (pre-Gondwana plant from N S W) 2. *L. nothum* Unger. (pre-Gondwana of N.S W) and 3. *L. aculeatum* in various respects.
* *Lycopodiopsis Derbyi* Renault (*Lepidodenron Derbyi* Renault)	1 Piracicaba, São Paulo 2 Tubarão 3 Iraty 4 Estrada Nova, and 5. Bofete all in Brazil	Permian	Renault, 1890; Zeiller, 1895, Arber, 1905, White 1908; Steinmann, 1924; Maack, 1929; Oliveira, 1937, and Roxo, 1938	Renault says that this may be the badly preserved stem of *Lepidodendron Pedroanum*, in which case the occurrence of the latter in pre-Gondwana beds gains added significance. White compares this closely with *Bothrodendron Leslii*
Lepidophloios laricinus, Sternberg	Brazil	with *Glossopteris* flora	Arber, 1905	The northern type occurs in Britain, France, Germany, Austria, Russia, N America and elsewhere (Upper Carboniferous)
Sigillaria Brardi, Brown	1 Do. 2 Vereeniging	1 " 2 Do (Lower) Permian Ecca series	Seward, 1897; White, 1908, Lundquist, 1919.	One specimen of *S. Brardi* from S Africa bears a close resemblance to *S Grasiana* Brongn from the Gard.
* *S. australis*	Brazil	With *Glossopteris* flora	White, 1908	
S. (?) *muralis*	Do	Do	Do.	
Sigillaria sp. Leaves	Do.	Do.	Seward, 1897; White, 1908, Lundquist, 1919	
Bothrodendron Leslii Seward	Lower Karroo at Vereeniging, S Africa.	Do.	Seward and Walton, 1923; White, 1908.	White compares this with *Lycopodiopsis Derbyi*.

5. *Summary*

Lycopodiopsis was founded by Renault as early as 1890 as a genus distinct from *Lepidodendron* but a doubt was thrown on the distinction between these two genera by Zeiller, who seems to have examined poorly preserved material. The present re-investigation definitely confirms Renault's view. The most important feature separating *Lycopodiopsis* from *Lepidodendron*, namely, the discontinuity of the xylem cylinder, is clearly seen in the stems here described and figured (Pl. X, Figs. 5–6).

This re-examination of *Lycopodiopsis* (which is said to be a typical member of the *Glossopteris* flora in South America) suggested a consideration of the southern *Permo*-Carboniferous lycopods in general. A definite decision on the difficult questions of the relation between the northern and southern lycopods and of the pre-Gondwana and Gondwana lycopods cannot be given. But as can be seen from the Tables, the majority of pre-Gondwana lycopods were cosmopolitan, though a number of them are exclusively southern. There is a great decline of lycopods in Gondwana times, but apart from two purely Gondwana lycopods, namely, *Bothrodendron Leslii* Sew. (Seward and Walton, 1923) and *Sigillaria* (?) *muralis* (White, 1908), there are some representatives of the so-called "northern" lycopods, which may perhaps with equal justification be regarded as hardy survivors (or more or less modified descendants) of pre-Gondwana forms.

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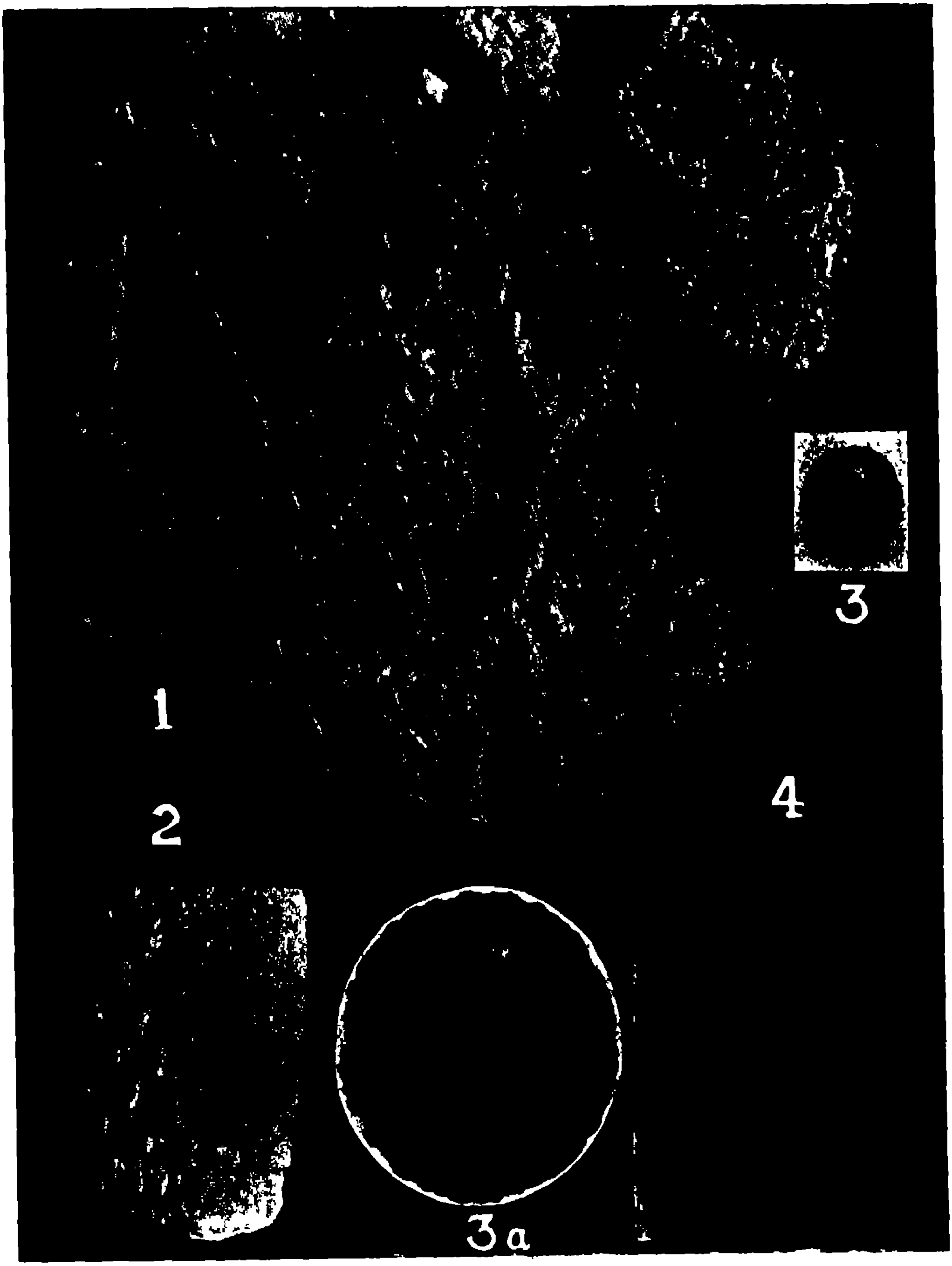
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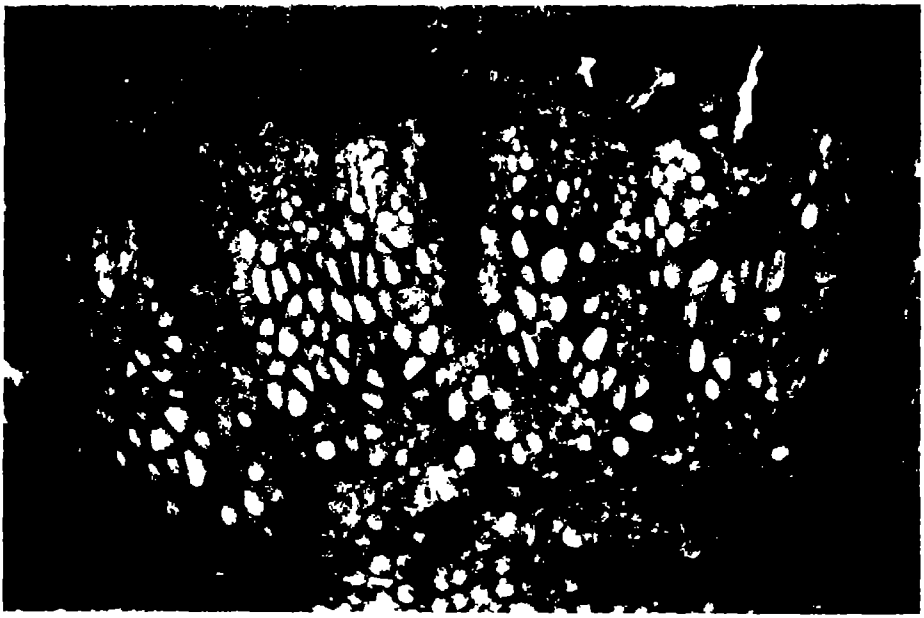

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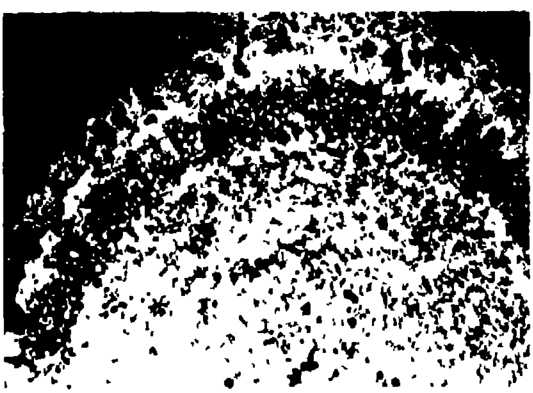

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EXPLANATION OF PLATES

Plate IX

Fig. 1. *Lycopodiopsis Derbyi*, from the Permian of the State of São Paulo An impression preserved in the Museum of Palæontology of the Geological and Mineralogical Survey of Brazil. Reproduced from Roxo (1938, Fig 15), in whose paper it was printed, it seems, upside down Nat size

Fig. 2. The Paris specimen . External features of the decorticated stem (compare Fig 1 for the external surface) × 2½

Fig. 3. The Paris specimen transverse section. × 1

Fig. 3*a*. The same magnified × 2½

Fig. 4. Annular or spiral tracheids of the leaf-trace in the leaf-cushion region from a transverse section of the Paris specimen × 300

Plate X

Fig. 5. The Bonn specimen A few xylem bundles of Steinmann's specimen highly magnified. Note the discontinuous bundles, the leaf-traces, the exarch protoxylems and the pith The dark patches in the pith, cortex and medullary rays may be secretory sacs × 435.

Fig. 6 The Paris specimen Part of the stele showing the discontinuous xylem ring, the pith and the exarch leaf-traces (near ends of black lines) consisting of a few elements. × 30

Fig. 7. The Paris specimen Longitudinal section of a leaf-cushion Note the vertically elongated cells and the absciss layer "a" × 11

Fig. 8 The Paris specimen A part of the transverse section enlarged to show the tissues of a leaf-cushion × 21.

A NOTE ON THE MORPHOLOGY OF THE ILIOFEMORAL LIGAMENT OF THE HIP-JOINT

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DURING the dissection of a female Indian Langur (*Semnopithecus entellus*) made for comparative study, a small muscle was noted on the antero-medial aspect of the hip-joint in intimate contact with the articular capsule. Its interest was increased when on reflection of the muscle it was found that the medial limb of the iliofemoral ligament was absent and the articular capsule in the position was very thin. A similar condition was noted also on the opposite side A second animal was subsequently dissected and it confirmed the original findings. The small muscle corresponds in situation to the *m. iliacus* minor (synonyms · *m iliocapsularis*, *m. iliotrochantericus*, *m iliocapsulofemoralis*). This muscle occurs sometimes as a human variation. But its normal occurrence in any monkey has not been previously noted. And at the same time the associated absence of the medial band of the iliofemoral ligament seemed also significant.

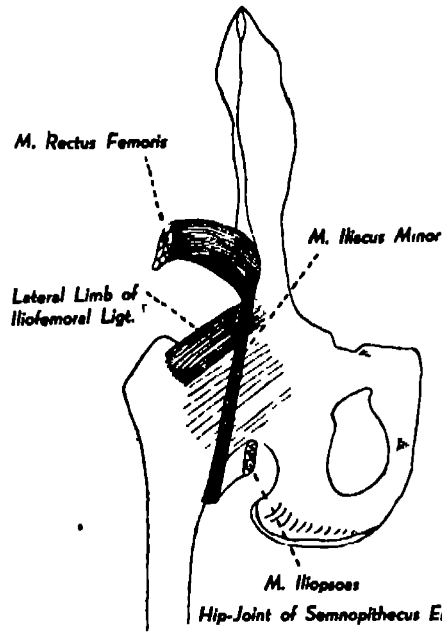
Observations

The *m. iliacus* minor in the *Semnopithecus entellus* is about half a centimetre in width and is purely muscular. Its origin is from the anterior border of the ilium above the acetabulum wedged in between the two heads of the *m. rectus femoris*. The point of origin is just above the attachment of the lateral limb of the iliofemoral ligament. The muscle is inserted to the lesser trochanter of the femur and at its insertion it is distinctly separable from the adjacent muscles. It is supplied by a twig from the femoral nerve.

The iliofemoral ligament of the hip-joint in *S. entellus* shows only the lateral limb extending from the lower part of the anterior iliac margin to the front of the greater trochanter. The medial limb is absent. The capsule is very thin in the antero-medial aspect and its distal attachment is to the femoral neck.

Comparative Anatomy of the M. iliacus *minor*

M. iliacus minor occasionally occurs in man (Bryce, 1923). It arises, when present, from the anterior inferior iliac spine and is inserted into the



Hip-Joint of Semnopithecus Entellus

lower part of the intertrochanteric line or iliofemoral ligament. Bardeen (1933) states that the lateral portion of the muscle iliacus arises from the ventral border of the ilium and is adherent to the direct tendon of the *m. rectus* femoris and the capsule of the hip-joint, and that it is sometimes more or less isolated, forming the muscle iliacus minor or iliotrochantericus.

The anthropoid apes do not show this muscle ; nor has it previously been described in any monkey Sonntag (1924) makes no mention of it in his book on the *Morphology of the Apes and Man* In the Macacus rhesus this muscle is not noted (Howell and Straus, 1933), though the iliofemoral ligament is said to be present (Sullivan, 1933) Duckworth remarks about a curious muscular slip he noted in a lemur, which was found winding spirally round the capsule of the hip-joint corresponding to the lower limb of the iliofemoral band. In the slender loris this muscle is not found.

The Morphology of the Iliofemoral Ligament

Keith (1933) holds the view that the anterior part of the capsule of the hip-joint has to withstand the strain of the body when the thigh is extended in the upright posture and that a part of it becomes specialised to form the iliofemoral ligament. This is a purely physiological explanation for its marked development. But many other authors have suggested muscular homologies for the iliofemoral ligament.

The lateral limb of the iliofemoral ligament has been said to be homologous with the muscle gluteus quartus or scansorius of the anthropoids (Sutton, 1887). Sisson (1935) describes a small muscle in the dog and horse called capsularis and says that it appears to represent the very strong iliofemoral ligament of man. But from the femoral attachment of the muscle capsularis in between the *m. vastus* intermedius and *m. vastus* lateralis, it is clear that the *m. capsularis* can only represent, if so, the lateral limb of the iliofemoral ligament. Rouviere's opinion is cited by Bryce (1915) suggesting the derivation of the iliofemoral ligament from the iliocapsulofemoral muscle. This muscle is the same as the muscle iliacus minor. Its position shows that it can only correspond to the medial limb of the iliofemoral ligament. Rouviere's view now gets a further support in the condition found in *Semnopithecus entellus*, where the muscle iliacus minor is present and the medial limb of the iliofemoral ligament is absent. It therefore appears very probable that the medial limb of the iliofermoral ligament is homologous with the iliacus minor muscle. Observations regarding the relative accentuation or feeble development of the medial limb of the iliofemoral ligament in the human subject in cases where the muscle iliacus minor occurs, as a variation, might be of value.

Summary and Conclusion

The muscle iliacus minor normally occurs in the *Semnopithecus entellus*. It takes origin from the anterior margin of the ilium between the two heads of the rectus femoris and is inserted into the lesser trochanter. The medial limb of the iliofemoral ligament is absent in this animal This adduces additional evidence for the view that the muscle iliacus minor and the medial limb of the iliofemoral ligament are homologous structures.

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A NOTE ON A CLUB-SHAPED VARIETY OF *BOTRYDIUM GRANULATUM* (L.) GREV. VAR. *CLAVAEFORMIS* VAR. NOV.

BY M S. RANDHAWA

Received December 16, 1939

THIS interesting variety of *Botrydium granulatum* was collected by the author from the highly manured sides of a pond near village Bhadrassa, District Fyzabad, on 15th January 1939 The sides of this pond are frequented by villagers for answering the call of nature, and human fæces which is converted overnight by insects into loose and well-manured soil provides a suitable substratum for the growth of terrestrial algæ A patch of this alga about 8 inches in width and 3 feet long was found in a drying water channel leading into the pond, which was well-shaded by a species of *Polygonum* The elongated vesicles of the alga which grows gregariously, resemble the pointed leaves of young moss plants

The young plants are almost filamentous in habit and are usually sinuous (Fig 1) They have blunt apices, and unbranched rhizoidal part below, and are 16–24 μ in diameter Slightly older plants are 25-84 μ in diameter, and their rhizoidal parts show a tendency towards dichotomous branching The subaerial vesicles are usually cylindrical or club-shaped in appearance and are very much elongated A tendency towards branching is seen in the subaerial parts of some of the younger plants (Fig 2)

The mature plants are usually club-shaped and are pointed at the top (Figs 3 and 4). The mature vesicles are 320 μ to 530 μ in diameter, their outer covering wall is fairly thin, and no encrustation of carbonate of lime was seen in any case Rhizoids are very richly branched in mature specimens (Fig 4) No cyst formation was observed

Affinities.—This alga is undoubtedly a variety of *Botrydium granulatum* (L.) Grev. from which it differs in the shape of its subaerial vesicles, which are spherical in typical specimens of *B. granulatum*, which too was collected from drying puddles at Fyzabad in August and September 1938 Iyengar also collected a form resembling the present alga in 1924 from Nandi Hills in Mysore; however, he gave no figures. In his brief preliminary description of that form he observes, " The second *Botrydium* mentioned above seems to be

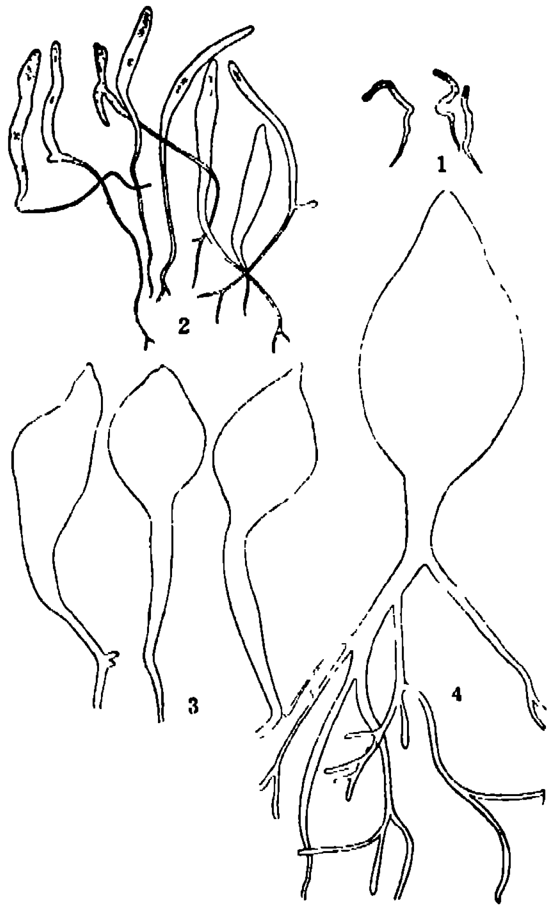

FIG. 1

Botrydium granulatum (L.) Grev. var. *clavaeformis* var. nov.

Fig. 1.—Three young plants. Fig 2.—A group of young plants. Mark the tendency towards branching seen in some of the plants. Fig. 3.—A group of middle-sized plants showing the subaerial portion only. Fig. 4.—A mature plant showing the club-shaped elongated subaerial part and branched rhizoidal part. All × 40.

a variety of *B. granulatum* and was collected by the author last December on the Nandi Hills in the Mysore Province Its upper green portion is somewhat elongated and very broadly conical at the top, the top portion ended though broadly in a point and is not rounded as in *B granulatum*. The cysts are formed in a row but at longer intervals than in *B. granulatum*."

The present form resembles the form collected by Iyengar in all necessary details. Its persistent club-shaped appearance warrants its establishment as a new variety of *B. granulatum*

Botrydium granulatum *(L.) Grev. var.* **clavæformis,** *var. nov.*

Mature plants club-shaped, apex pointed, 320–530 μ in diameter, outer wall thin Young plants more or less filamentous, with subaerial parts occasionally branched

Habit.—Found growing at the sides of a pond near village Bhadrassa, District Fyzabad, U P , India, mixed with *Vaucheria geminata* on 15th January and 17th February, 1939

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EXPERIMENTAL STUDIES ON DIETS DEFICIENT IN VITAMIN B AND THEIR INFLUENCE ON THE INTESTINAL YEAST FLORA OF ANIMALS

By Prof. Col. I. Froilano de Mello (Nova-Goa)

Received April 29, 1940

Introduction

The widely spread blastomycosic theory of human sprue has been for us the primary reason for suggesting experimental studies to enquire whether some relation may be found between the yeast contents of animal intestines and diets deficient in some kind of vitamins. So, it will not be devoid of interest to summarise, first of all, the different views concerning the ætiological relations between yeast and sprue.

These views may be grouped under three main lines :

1. The intestinal yeasts are the true cause of sprue

Ashford claims his *Monilia psilosis* (= *M. ashfordi*) as the cause of the disease[1]. This view is so strongly advocated that Manson Bahr changes the clinical name *sprue* into *psilosis*[2]. Ashford finds in the cure or amelioration of sprue patients through specific monilia vaccines new arguments for this theory[3]. Johnson and Breidigain confirm this fact in a patient[4]. Smith attributes to yeasts the anemia in sprue[5], which according to Wood should be due to an hemolytic toxin[6], as seen in experimental intraveinous injections made by the same author in rabbits with filtrates of *M. psilosis* in dextrose.

The monilia theory has found such a credit that some of its partisans believe that the discovery of *M. psilosis* in stools can be utilised as a differential diagnosis test between biermerian and spruic anemia[8]. Garcia and co-workers isolate *M. psilosis* in 9/10 of spruic patients.[9]

2. The so-called blastomycetic nature of sprue, be it due to *M. psilosis* or any other species of yeast, is entirely rejected.

Weiss and Landron[10] do not admit *M. psilosis* as the cause of sprue. In India, Fairly and Mackie find this Monilia indifferently in spruic and non-spruic patients[11], and do not succeed to reproduce the disease in monkeys through *Monilia* injection—(note that the authors found a "*ashfordi type*"

yeast in the intestine of normal monkeys). Mackie and Chitre confirm these results in 1928[12] and are unable to reproduce the sprue in animals fed with human intestinal monilia[13]. Fairly and Jasudasan preparing an anti-yeast serum show that antibodies are not found in sprue patients[14]. Van Steenis studying this disease in Java, denies to *M. psilosis* any action in the ætiology of sprue.[15]

3. Intestinal yeasts, while not being the true cause of sprue, are not however devoid of some secondary morbid action.

In this group we find the most varied opinions : A le Dantec describes in sprue two phases, the first one of an acid diarrhea due to paralactic bacilli, the second one due to a symbiosis of these bacilli with yeasts[16]; Manson Bahr considers them as secondary invaders in any debilitating diesease[17]. Efremow[18] does not consider *M. psilosis* and his *M. armeniensis* as harmless symbionts, Elders[19] points out them as agents of secondary infections. De Mello[20] believes that the intestinal yeasts are not the cause of sprue but that they represent in sprue and spruic stages, a test of acidosis and of a certain degree of avitaminosis concerning probably a deficiency in flavines.

It would be impossible to quote the whole literature on the subject. Reference will however be made to the researches of Weld Smith who, submitting guinea pigs to a scorbutic regime and feeding them afterwards with cultures of *M. psilosis* succeeded to develop thrush in their buccal cavity[20].

Our actual experiments concern only diets deficient in vitamin B through feeding the animal with cooked white polished rice, so largely used by the rural folk in India and will be followed by other types of deficient nourishments, in order to complete the proposed scheme on the relations between avitaminosis or hypovitaminosis and the yeast contents of animal intestines.

Part I

1. *Experiments with white rats*—(in collaboration with my pupil Jonas de Sa Viegas).

The experiment began on 1-11-39 and ended on 10-12-39. Animals of contrôle are labelled N (normal); those submitted to experimental diet, E (experiment). All in separate cages.

(a) *Weight of the white rats during the experiment (in grams)*

	1-11-39	10-11-39	21-11-39	28-11-39	REMARKS
N 1	136	..	117	.	The experimental feeding ended on 10-12-39 and the weights stated in the last column were taken 17 days after return to normal regime. The weight has suffered even in contrôles many variations which do not give room for a systematisation. No rat has shown definite paralysis. Evident signs of hypovitaminosis (animal sluggish, bristled up hairs) have been however noticed between 12th to 15th day, in all rats submitted to experimental regime
N 2	104	.	107	..	
N 3	102	..	93	.	
N 4	103	.	105	..	
N 5	72	..	59	..	
E 1	148	122	151	154	
E 2	186	166	170	170	
E 3	148	157	148	154	
E 4	139	114	109	112	

(b) *Yeast count in* 1/10 *c.c. of an homogene solution of fæces*

1. *In controls.*

		REMARKS
N 1	7	Having seen that the degree of this infestation varied to such a large extent, four more rats were examined with the same technique and the following results obtained :
N 2	7	
N 3	9	N 6 1566
N 4	20	N 7 429
N 5	312	N 8 528
		N 9 uncountable

2. *In experiment animals* (all of them completely separated one, from another, in distinct cages to avoid the possibility of any intercontamination).

	Before the exp.	10 days after the exp.	40 days after	20 days after returning to normal regime
E 1	20	260	569	719
E 2	429	923	1298	1109
E 3	885	1360	uncountable	1202
E 4	528	909	1165	572

Summarising the statements :

1. All the white rats, either control or under the experiment, show in their stools yeast flora whose degree of infestation vary extremely from one individual to another.

2. The rats under the experiment showed 10 days after the deficient beriberigen regime a definite increase in the degree of this infestation, increase which continued till the end of the experiment.

3. The animals under experiment after their return to the normal regime, showed an uncontestable decrease in the yeast infestation of their intestinal contents (analyses made 20 days after the return), without, however, reaching the primitive ratio, excepting in E 4 where the latter number was nearly the same as the former.

2. *Experiments with hens*—(in collaboration with my pupil Armando Baptista Cardoso).

The experiment began on 21-11-39. On 14-12-40 definite polynevritis gallinarum having been noticed, all the animals were put under normal regime on 16-12-40.

Control animal labelled N (normal). Those under experimental feeding E. All in separate cages.

Weight of the animals during the experiments (in grams)

	Before the exp.		During the exp.		After return to normal food		REMARKS
	6–11–39	20–11–39	12–12–39	14–12–39	27–12–39	22–1–40	
N	916	855	787	557	681	815	Are given below
E 1	980	976	868	729	954	855	
E 2	770	744	659	544	647	755	
E 3	803	915	886	738	..	..	
E 4	860	840	714	546	708	905	
E 5	890	950	887	692	787	1045	

REMARKS

N Confined in the cage, does not eat well. Paresia since 7–12–39. Frank polynevritis gallinarum on 14–12–39. Injection of betaxin, the animal is free. Cure on 27–12–39.

E 1 Beriberi on 14–12–39, aggravated on 16–12–39. Injection of betaxin.

E 2 Beriberi on 16–12–39. Immediately put into normal regime and in liberty, but the symptoms worsening. Betaxin on 22–12–39, again betaxin on 27–12–39.

E 3 Beriberi on 14–12–39. Put in liberty, died under a motor car.

E 4 and E 5 Beriberi on 14–12–39. Put in normal regime and liberty on 16–12–39. Cure without betaxin injection.

Yeast count in 1/10 *c.c. of an homogen solution of stools*

	Before the exp.	During the experimental feeding		After return to normal food		
	9–11–39	7–12–39	14–12–39	27–12–39	4–1–40	22–1–40
N	uncountable	rare	rare	140	397	398
E 1	867	689	rare	138	412	494
E 2	uncountable	178	rare	96	281	uncountable
E 3	670	1504	127	..	..	..
E 4	394	2046	rare	uncountable	uncountable	487
E 5	593	1409	rare	160	462	uncountable

Summarising the statements :

1. All the animals showed, between 22 to 24 days after beginning the beriberigen feeding, a typical polynevritis gallinarum. In control hen, this disease developed 7 days earlier, but it must be emphasised that this animal refused practically the food.

2. Polynevritis was noticed after a certain loss of weight whose degree can be calculated, in every case, according to the numbers quoted in the table above (the weight recorded in the last two columns was taken after the return to normal food, that of the last one being when the animals were put in liberty)

3. All the animals have in their intestines yeasts whose number in normal stage of health varies according to the individuals.

4. The first analysis made during the experiment, 16 days after the beginning of the beriberigen feeding, showed variations in the degree of yeast infestation, impossible to be systematized.

5. Curious to remark, at the outset of the first symptoms of polynevritis, a marked diminution in the number of yeasts is noticed, in contrast with a slight increase when these symptoms were attenuated or disappeared (even in N, where polynevritis was noticed 7 days before the other hens, the same phenomenon occurred).

6. After return to normal feeding the yeast infestation degree increased and showed individual variations impossible also to be systematised.

7. The curious decrease stated in No. 5 together with the fact already known of the antiberiberic action, for instance, of the beer yeast, suggests the possibility of considering the intestinal yeasts of hens as possessing defensive properties destined to delay the avitaminic syndrom—a point which requires further investigation.

3. *Experiments with rabbits*—(in collaboration with my pupil Miguel A. da Costa).

The experiment began between 28 to 30-10-39 and ended on 21-12-39. Control rabbit labelled N; those under experimental regime E.

Weight of the animals (in grams)

	Before the expt.	During the expt.			
	26–10–39	14–11–39	30–11–39	7–12–39	21–12–39
N	1300	1297	..	1253	1244
E 1	1270	1255	1219	1170	1155
E 2	1300	1259	1230	975	915
E 3	1350	1312	..	..	1272
E 4	1397	1359	1297	..	1108

Yeast count in 1/10 *c c. of an homogeneous solution of stools*

	26–10–39	30–10–39	12–11–39	17–11–39	7–12–39	21–12–39
N	6	17	21	27	66	68
E 1	157	179	215	.	243	276
E 2	189	..	546	531	526	544
E 3	246	309	530	537	347	231
E 4	117	.	256	..	275	325

Summarising the statements :

1. All rabbits showed their weight decreased, but while in control animal this loss was only of 54 g./1,300, the animals E 1, E 2, E 4 lost respectively 115/1240, 385/1300, 289/1397. The rabbit E3 lost, however, only 78/1350.

2. While the control rabbit remained normal, those under the experimental regime showed : (*a*) paresia of hind members; (*b*) general weakness; (*c*) diarrhea (E 2, E 3).

3. All rabbits have normally yeasts in their intestinal contents. Under the deficient regime there is a certain increase in the degree of this infestation. Only in E3, after this increase, there is a sudden diminution, the last

analysis giving a number slightly inferior to that of the normal state (interesting to note that the increase corresponds to the period of diarrhea, from which the animal was afterwards free).

4. *Experiments with pigeons*—(in collaboration with my pupil L. Sales d'Andrade e Souza).

The experiment began on 4–11–39. On 24–11–39, the pigeon E 1 having shown a definite opisthotonos, an injection of betaxin was given and all pigeons put in normal diet. Control pigeon labelled N; those under experimental regime E.

Weight of the animals (in grams)

	26–10–39	24–11–39
N 1	325	321
N 2	295	299
E 1	272	212
E 2	312	286
E 3	260	208
E 4	271	196

Yeast count in 1/10 c.c. of an homogeneous solution of stools

	Before the experiment	At the end of experiment	After return to normal regime		
	26–10–39	24–11–39	27–11–39	30–11–39	12–12–39
N 1	392	363	253	236	228
N 2	364	306	269	218	276
E 1	704	1632	644	629	495
E 2	192	850	755	458	550
E 3	481	1284	574	495	466
E 4	uncountable	504	343	245	345

Summarising the statements :

1. Pigeons submitted to a beriberigen regime showed loss of weight already known to all pathologists and one of them polynevritis, 20 days after the beginning of the experiment, the others having been immediately put under normal regime.

2. All pigeons show normally yeasts in their intestinal contents.

3. While in control pigeons the number of these yeasts shows small variations, in those under experiment, it increases enormously, and suffers a gradual and progressive reduction when the animals are again put in normal regime.

4. Only pigeon E 4 showed a very strong infestation before the experiment, but it must be stated that at the date this pigeon had a green diarrhea and it is quite possible that the enormous number of yeasts recorded in the first analysis is due to this accidental cause. Anyhow, the subsequent analyses, after return to normal feeding, show a progressive reduction, with a slight increase in the last column (it is to be regretted that between 26–10-39 to 24–11–39, no record was taken, which could be useful for a comparison).

Summary and Conclusions

1. White rats, hens, rabbits and pigeons have been submitted to a beriberigen regime, constituted by white polished rice. After the appearance of symptoms of B avitaminosis or hypovitaminosis, they have been returned to the normal feeding.

2. The analysis of the stools of these animals shows that all of them possess in their intestine, even in normal health, yeast flora more or less abundant according to the individuals.

3. Generally speaking, under deficient beriberigen regime, the number of intestinal yeasts increases and the more the animal is weak, larger is the number of the yeasts. This experimental fact may give an explanation to the appearance of levurotic complications in organisms rendered debile through disease, exhaustion or some other causes.

4. After putting the animals suffering from experimental avitaminosis of hypovitaminosis in normal regime, the number of intestinal yeasts generally decreases to the level of the normal stage of health.

5. The curious phenomenon noticed in hens, that on the eve of appearance of definite avitaminosic symptoms, the number of yeasts falls down abruptly and is followed by gradual increase when the animal recovers, does not, in our opinion, constitute an exception to the statement contained in

para 3. Combined with the fact already known of the antiberiberic action of some yeasts, it suggests rather the possibility of considering these yeasts as a defensive factor delaying the appearance of the B avitaminosis. This point requires further experimental investigation.

6. It has been noticed in pigeons that an eventual stage of diarrhea increases enormously the number of intestinal yeasts.

7. Our general conclusion is that the yeast infestation of the intestines of animals reported above should be considered as a case of normal commensalism; the variations in the degrees of such an infestation may however be taken as an analytic test for deficiency arisen from beriberigenic diets.

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DIURNAL MARCH OF CARBOHYDRATES IN RELATION TO BIOCHEMIC CONSTITUTION OF LEAVES

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Introduction

IT has been recently realised that out of a complicated series of interlinked carbohydrate transformations usually taking place in the assimilating region, the cell maintains a sort of "unidirectional carbohydrate flux" in virtue of which some assimilates accumulate in larger aggregate than others. Brown and Morris,[2] Gast,[5] Parkin[11, 13] and Miller[10] have shown that the percentage of hexoses in leaves remains fairly constant throughout the course of the day whereas sucrose fluctuates markedly increasing by day and decreasing by night. Dixon and Mason[4] have demonstrated the presence of hexoses in chloroplasts and suggested that the hexose molecules are condensed to sucrose which acts as a temporary reserve. Davish, Daish and Sawyer[3] investigated the hexose sucrose ratio in veins and lamina and concluded from their observation that sucrose was the primary sugar formed. Kylin,[6] Priestley,[14] Mason and Maskell,[8] and Weevers,[23] have also studied the formation of carbohydrates in plants but the problem of first carbohydrate originating from photosynthetic reduction of carbon dioxide remains still unelucidated, resting as it does, upon the consensus of evidences obtained by manifold experimentation.

Such an analysis of the problem of first product of photosynthesis has been rendered difficult partly due to the complexities introduced by the condensation of simpler upgrade carbohydrates to more stable molecules and partly due to the hydrolysis of highly complex starches, inulin and polysaccharides to simpler downgrade sugars. Their synthesis, accumulation and translocation, simultaneously or otherwise, the subtle changes that are prone to take place in their relative concentrations, and the absence of good methods of estimating their percentages, have made the issue much more complicated.

In the studies to be reported in the present communication an attempt has been made to investigate the march of carbohydrates in a number of

tropical plants where certain well-marked peculiarities in functional activities in contrast to those observed in temperate regions have already been recorded.[16-21] Due consideration has been given to the factor for translocation, the simultaneous accumulation of products other than carbohydrates, and the critical concentration of simpler sugars required to induce the synthesis of starches in leaves belonging to different genera, species and varieties.

Experimentation

To this end in view a large number of plant species including *Ricinus communis*, *Brassica alba*, *Linum usitatissimum*, *Pisum sativum*, *Cicer arietinum*, *Cajanus indicus*, *Phaseolus vulgaris*, *Triticum vulgare*, *Oryza sativa*, *Zea Mays*, *Solanum tuberosum*, *Hordeum vulgare*, *Saccharum officinarum*, *Allium cepa*, *Beta vulgaris*, *Gossypium neglectum*, etc., were grown under conditions of optimum soil nutrition at the Experimental Farms attached to this Research Station. Two series of picking of leaves for chemical analysis were done. In the first instance, mature leaves were gathered from average healthy plants at successive hours of a clear day while in the second a large number of detached leaves from such plants were picked up only in the evening and kept overnight in the laboratory under suitable conditions of moisture. The leaves of second series were then graded into a number of groups each one of which was exposed for varying periods to artificial illumination from electric lamp fitted with parabolic reflector and giving an illumination intensity of 80,000 metre candles. At regular intervals one of such sets was removed and the plant material fixed in five per cent. formaldehyde. The leaves so treated were carefully washed, the adhering water wiped off, the fresh weight recorded and finally the leaves incubated to constant weight in a ventilated steam oven regulated at 80° C. after the method of Link and Tottingham.[7] The presence of starch was tested by the well-known micro-iodine method. The iodine solution was prepared by dissolving 1 gm. of iodine in 100 c.c. of five per cent potassium iodide solution to which 5 gm. of chloral hydrate was added to clarify the section. Grape sugar, cane sugar, and maltose were used for these feeding experiments. For details of the method of analysis of carbohydrates, proteins and fats reference may be made to " Official and Tentative Methods of Analysis ".[22]

Results

The percentage of glucose, sucrose and starch in attached leaves of *Triticum vulgare* and *Saccharum officinarum* gathered at different periods of the day increases with an increase in the period of illumination till 2 P.M. and subsequently exhibits a decline irrespective of the continued exposure

of leaves to favourable light conditions for several consecutive hours. Such a characteristic drift with an afternoon maximum is specially noted in the case of glucose and sucrose whereas in case of starch the maximum is not reached before 8–10 P.M. Soon after the attainment of a maximum, the carbohydrate percentage exhibits a characteristic decline probably brought about by the translocation of these substances to the leaf sheath and stem and their utilisation during downgrade metabolism or otherwise.

A further analysis of the data reveals that the percentage of different carbohydrates in general varies with the type of the experimental material. To take an instance, it is found that the maximum percentages of glucose, sucrose and starch are 2·88, 8·89 and 20·69 in case of *Triticum* sp., (Table I) and 2 96, 10·85 and 20·32 respectively, in case of *Saccharum* (Table II).

TABLE I

Percentage of carbohydrates calculated on dry weight basis in attached leaves of Triticum vulgare, 19th August 1933

Time	Glucose	Sucrose	Starch
4 A.M. .. .	1·03	4·33	16·770
6 "	1·14	4·91	14·320
8 " .. .	1·45	5·42	14·980
10 "	2·24	6·78	15·680
12 Noon . .	2 68	8·51	14·966
2 P.M. . .	2·88	8·89	15·992
4 "	2·62	8·66	18·461
6 "	1·95	7·29	18·831
8 "	1·64	6·81	20·690
10 "	1·49	6·10	20·650
12 Midnight .	1·26	5·26	18·234
2 A.M.	1·24	5·03	17·683

TABLE II

Percentage of carbohydrates calculated on dry weight basis in attached leaves of Saccharum officinarum, 19*th August* 1933

Time	Glucose	Sucrose	Starch
4 A.M.	1·19	6·230	14·422
6 „	1·94	6·910	13·635
8 „	1·98	8·420	14·261
10 „	2·45	9·781	16·770
12 Noon . ..	2·96	10·851	17 045
2 P.M.	2·96	10·221	18·747
4 „	2·32	9 109	18·978
6 „ .. .	1·96	8·924	19·423
8 „	1·32	8·023	20·301
10 „ . .	1 43	7 651	20·320
12 Midnight ..	1·36	7·660	18·441
2 A.M.	1·24	6·480	16·627

It is remarkable to note that the relative concentration of complex carbohydrates accumulating in leaves appear to be more than those of simpler ones, although the latter on all grounds precede the synthesis of the former. This apparently suggests that the complex carbohydrates accumulate at the cost of simpler ones, an increase in the concentration of which beyond a certain range results in the formation of the starches.

The leaves picked up before sunrise also show the presence of carbohydrates although a considerable portion of the same disappears during night. Sucrose percentage decreases to the greatest extent while those of reducing sugars and starch diminish to a lesser degree. It is a matter of great significance that none of the workers in this line have found the absence of reducing sugars in the leaves. Even Brown and Morris[2] who declared glucose to be absent towards the end of the day could not do so

with regard to reducing sugars as a whole, the presence of lavelose being unavoidable in the experimental leaves.

In the variegated leaves of *Acalypha* sp., the ratio hexose/sucrose is considerably higher in the yellow regions as compared to the green regions, a result, which goes against the findings of Weever.[23] Cassia leaves (Table III) on short exposure to morning light give increased hexose and

TABLE III

The carbohydrate content of leaves calculated on dry weight basis exposed to shorter periods of illumination

Leaf	Picking	Percentage dry-weight basis			Hexose/Sucrose
		Glucose	Sucrose	Starch	
Artocarpus integrifolia	5–00 A.M.	1·23	0 02	2·40	*a*
	7–15 „	1 63	0·37	4·60	4·40
Cassia fistula	5–15 „	1·26	0·01	2·62	*a*
	8–00 „	1·44	0 005	3·79	*a*
Gossypium neglectum	5–00 „	0 93	0 004	2·125	*a*
	8–30 „	1 22	0·13	2·41	8·77
Acalypha sp.— Yellow region .	8–30 „	2·54	0·02	3·71	127·00
Green region .	8–30 „	3·40	0·59	6·41	5·76

starch but strange to say no sucrose. On these grounds, therefore, neither cane sugar can be claimed to be the first sugar of photosynthesis (Cp. Kylin[6]) nor is it possible to identify the hexoses as the first product of photosynthesis of fairly solid grounds. The investigations of Brown and Morris[2] Gast[5], Davish, Daish and Sawyer,[3] Parkin,[11–13] Priestley[14] and others also indicate that it is rather difficult to ascertain as to which is the first sugar formed.

The fluctuations in the carbohydrate content of detached leaves are also of practically the same order as that obtained for attached ones with the difference that the relative percentage of glucose, sucrose and starch is

different when compared to those recorded above (Table IV). In spite of the fact that the factor of translocation has been reduced to nil and that the

TABLE IV

Percentage of carbohydrates, proteins and fats in the detached leaves of Saccharum officinarum exposed to varying periods of artificial illumination

Hours of fixing the material	Duration of illumination	Percentage dry-weight basis				
		Glucose	Sucrose	Starch	Proteins	Fats
7 P.M. ..		2·84	14·72	14 45	18·20	6·42
4 A.M. ..	9 hrs. in darkness	1·24	11·42	12·21	10·65	7·89
6 „ .	2 hours in light	1·63	13·86	13·91	11·28	7·02
12 Noon ..	8 „ „	2·87	19·24	14·42	13·06	6·59
6 P.M. ..	14 „ „	2·72	18 02	14 00	18·81	7·02
12 Midnight	20 „ „	2·69	17·56	13·68	10·18	7·93
4 A.M. ..	24 „ „	2 45	14 56	12·31	9·80	8·38

conditions of assimilation are quite favourable, the percentage of various carbohydrates decline after the attainment of a maximal hump. Such a maximum concentration, upto which the leaves can hold the different carbohydrates, is identified with 2·87, 19·24 and 14·42 per cent. of glucose, sucrose and starch respectively (Table IV), an increase beyond which probably results in the formation of some other products or their utilisation in some other ways independently. The accumulation of fats and proteins in leaves exposed to light and their characteristic diurnal march gives substantial evidence to the effect that such complex substances are also formed in assimilating leaves simultaneously with carbohydrates and that in consequence they have some direct or indirect relationship with the upgrade metabolism of plants.

The data obtained in this connection (Tables IV, V, VI, and VII) also lead us to the view that of all the experimental plants, *Saccharum* accumulates in its leaves the maximum percentage of sugars—glucose and sucrose

TABLE V

Percentage of carbohydrates, proteins and fats in the detached leaves of Oryza sativa exposed to varying periods of artificial illumination

Hours of fixing the material	Duration of illumination	Percentage dry weight basis				
		Glucose	Sucrose	Starch	Proteins	Fats
7 P.M. ..		2·38	5·94	22·65	14·00	2·32
4 A.M. ..	9 hrs. in darkness	1·09	3·21	20·32	13·12	7·45
6 „ ..	2 hours in light	1·33	4·68	21·52	17·41	5·77
12 Noon ..	8 „ „	2·46	6·98	22·73	13·12	5·60
6 P.M. ..	14 „ „	2·40	6·62	22·80	11·76	5·90
12 Midnight	20 „ „	2·39	5·42	21·43	10·69	6·30
4 A.M. ..	24 „ „	1·96	5·39	20·78	10·21	6·81

TABLE VI

Percentage of carbohydrates, proteins and fats in the detached leaves of Phaseolus vulgaris exposed to varying periods of artificial illumination

Hours of fixing the material	Duration of illumination	Percentage dry weight basis				
		Glucose	Sucrose	Starch	Proteins	Fats
7 P.M. ..		2·03	4·41	10·55	20·36	8·37
4 A.M. ..	9 hrs. in darkness	1·66	2·83	8·82	17·60	9·24
6 „ ..	8 „ „	1·83	3 96	9·24	21·98	8·68
12 Noon ..	8 „ „	2·25	5·68	9·93	19·26	7·96
6 P.M. ..	14 „ „	2·02	4·73	9·10	24·87	8·53
12 Midnight ..	20 „ „	1·96	3·59	8·65	12·69	9·00
4 A.M. ..	24 „ „	1·28	2·29	8·01	11·40	9·92

TABLE VII

Percentage of carbohydrates, proteins and fats in detached leaves of Ricinus communis exposed to varying periods of artificial illumination

Hours of fixing the material	Duration of illumination	Percentage dry weight basis				
		Glucose	Sucrose	Starch	Proteins	Fats
7 P.M. ..		2·04	4·10	9·34	18·83	10 68
4 A.M. ..	9 hrs. in darkness	1·16	3·21	6·82	16 62	12 84
6 " .	2 hours in light	1 80	4·16	7·46	19·04	12·42
12 Noon ..	8 " "	2·23	5·11	8 93	18·99	10·64
6 P.M. .	14 " "	2·06	4·67	8 50	20·05	13·00
12 Midnight	20 " "	1·80	4·32	8 30	20·34	13·96
4 A.M. ..	24 " "	1·65	4 02	8 00	17 25	14·42

while *O. sativa*, *R. communis* and *P. vulgaris* have the highest concentration of starch, fat and protein respectively, as compared to other products accumulating in assimilating leaves. The economy of these products also appears to be correlated with the final biochemic products which these very different species store towards the close of their life-cycle. Thus the sugarcane leaves accumulating sugars more than other substances also store such simple carbohydrates in the stem towards the maturity period Similarly the storage of starch in *Oryza*, proteins in *Phaseolus*, and fats in *Ricinus* towards the close of the life-cycle when taken in conjunction with the economy of similar materials in their assimilating leaves, gives indication to the fact that there is some sort of biochemic specificity in the products accumulating in leaves as well, in virtue of which the assimilating cells have the tendency to hold some substances in larger aggregates than others.

To test the possibility of the accumulation of such complex substances in leaves at the cost of simpler sugars as well as to investigate the critical concentration,* if any, of such sugars required to induce their accumulation,

* The criterion of determining such critical concentration has been the initiation of starch synthesis as indicated by the iodine test. Critical concentration is thus that minimum concentration of the external solution at which the starch molecules are just formed in the completely starved leaves floated on sugar solution.

a series of other feeding experiments were conducted. Starch synthesis in completely starch-free foliage was mainly investigated in darkness under varied concentration of certain sugars. It may be mentioned in this connection that the time required by different species of plants to remove the last traces of starch under complete dark conditions differs in a very characteristic manner. Leaves gathered from plants of oily constitution take a longer period to completely starve themselves as compared to those of proteinaceous, starchy or sugar groups. The more complex the biochemic nature of the accumulates, the longer is the interval required to remove the last traces of starch (Table VIII). It may also be observed that plants could

TABLE VIII

Critical concentration of sugars required to induce starch formation and time taken by leaves to become completely starch-free

Leaf materials	Time taken to become starch-free (in hours)	Critical concentration in gram-molecule		
		Glucose	Sucrose	Maltose
Cana indica	238	0·005	0·004	0·003
Gossypium neglectum	166	0·010	0·008	0·005
Eucalyptus sp.	161	0 025	0·015	0 012
Ricinus communis	118	0·010	0·008	0·008
Crotalaria juncea	100	0·020	0·015	0·003
Cajanus indicus	96	0·020	0·008	0·006
Oryza sativa	80	0·020	0·005	0·001
Zea Mays	78	0·050	0·020	0·015
Tagets erecta	76	0·008	0·008	0·003
Erythrina erista Galli	74	0·005	0·004	0·002
Mangifera indica	64	0·015	0·003	0·003
Cucumis sativus	74	0·015	0·005	0·002
Saccharum officinarum	60	0·010	0·005	0·003

as well be grouped with respect to the range of concentration of sugars that they require for starch synthesis in darkness. But there appears to be no specific relation between the inherent nature of the plant material and the critical concentration of sucrose, glucose or maltose required for inducing starch formation.

It is to be further noted that the critical concentration of glucose in the different types of plants described above when compared with the corresponding critical values in case of sucrose and maltose are found to be considerably higher than the other two. There appears to be a definite critical concentration for different sugars beyond which any increase usually results in the synthesis of starch The data collected in this direction, however, do not justify the conclusion drawn by Meyer[9] that dicotyledons in general require on an average lower concentration of sugars for starch formation than monocotyledons nor do they support the view held by Parkin[11-13] that maltose has little effect on starch synthesis.

Further in view of the fact that substances of so complex a nature as proteins and fats are also formed in assimilating leaves, it would indeed be interesting to investigate in its greater details whether the synthesis of such complex bodies too could be induced in darkness in presence of certain sugars, and if so, whether there is any specificity with regard to the critical concentration of sugars that may be necessary to induce their formation. This would further throw light on the question of biochemic constitution of plants thus giving us an idea of the possibilities of the segregation of plants into different groups on the basis of their biochemic characteristics as indeed the facts recorded by Reichert[15] and reviewed by Blackman[1] lead us to observe.

Summary and Conclusions

The present paper deals with the diurnal march of carbohydrates in leaves gathered from plants of varying structural and biochemic constitution with special reference to the formation of glucose, sucrose, starch, proteins, and fats. The experiments were conducted on attached and detached leaves both under artificial and natural illumination.

The synthesis of starch in completely starch-free leaves under complete darkness has been studied by performing sugar feeding experiments with a view to determining the critical concentration of sugars required for inducing starch formation.

The different carbohydrates—glucose, sucrose, and starch—have more or less similar diurnal march, although variations are but apparent in their absolute quantities as well as the period when their maximum concentration

in leaves is attained. Glucose and sucrose in general, attain their maximum in the leaf earlier than starch. The saturation limit for each of these constituents varies with the plant.

Absolute increase in the percentage of complex carbohydrates like starch, is greater during a given period of assimilation when compared to simpler carbohydrates like glucose or sucrose. It seems that as soon as the percentage of sugars increase beyond a certain concentration, the excess is converted into complex carbohydrates like starch.

The yellow regions of assimilating leaves give smaller quantities of sucrose in proportion to hexose, while in green parts both these sugars increase side by side. The hexose/sucrose ratio is thus greater in the first case while less in the other. The possibility of sucrose being more connected with photosynthesis is, however, not fully borne out by the many-sided experimental observations.

Side by side with an increase in carbohydrates an increase in the fat and protein content of assimilating leaf also takes place. The degree of economy for these different products in leaves exposed to illumination however varies with the species under experimentation, and appears to be correlated with the biochemic nature of the final products that the species store towards the close of their life-cycle.

The time taken to completely starve different species and varieties has been shown to depend upon the biochemic constitution of the plant material. The more complex the biochemic nature of the accumulates, the longer is the interval required to remove the last traces of starch.

The critical concentration of different sugars required to induce starch synthesis under darkness varies with the plant material as well as the nature of the sugar molecule.

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ON TYROSINASE OF *DOLICHOS LABLAB*

I. Methods of Estimation and the Oxidation of Different Substrates

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THE presence of an active tyrosinase in the aqueous extract of *Dolichos lablab*, was observed by Narayana in the course of his work on the proteins of Indian pulses. The enzyme was subsequently investigated by Narayanamurti and Iyer.[1]

Preparation of the Enzyme.—The enzyme is prepared by an aqueous or saline extraction of the dried and powdered seeds of *Dolichos lablab*, the extract after filtration being dilaysed to remove globulins. The clear, filtered, light brown solution was fractionally precipitated with ice-cold alcohol. The precipitate between alcohol concentrations 20–60%, was recovered by centrifugation and dissolved in water. The solution after further dialysis, has been employed in the following studies.

In attempting to prepare the enzyme in a state of high purity, the adoption of a reliable method for the quantitative estimation of its activity, is essential. Chemical methods now in vogue, take advantage of the disappearance of tyrosine during the oxidation or of the formation of quinone as the reaction product. The manometric method of determining the O_2 uptake has also been employed. O_2 uptake accompanying the oxidation of a suitable substrate affords a convenient means of measuring the activity of the enzyme preparations.

Rate of Disappearance of Tyrosine during Oxidation by the Enzyme.—This method was developed by Raper,[2] and improved considerably by Haehn and Stern.[3] It was employed by Narayanamurti and Iyer[1] in their studies of the kinetics of the tyrosine-tyrosinase reaction. The method consists in the estimation of the tyrosine, left after removing the enzyme and proteins and the melanin-like oxidation products by suitable treatments from an aliquot of the reaction mixture, by bromination with bromate-bromide mixture. We find that the curves representing the rate of disappearance of

tyrosine for various concentrations of the enzyme (Fig. 1) hold no measurable relation, sufficiently accurate to serve for an evaluation of the activity of the enzyme.

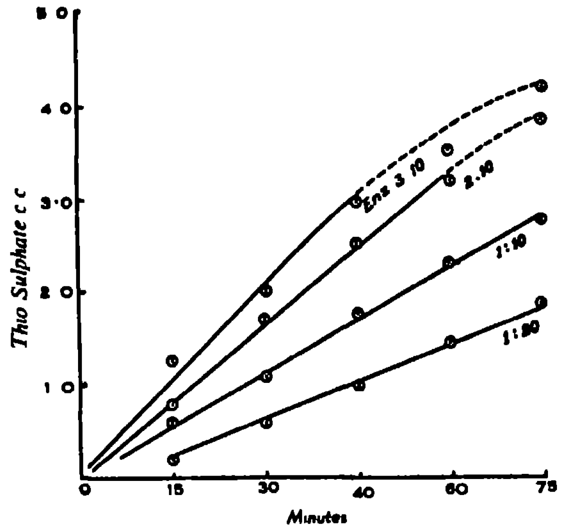


FIG 1
Tyrosine Rate of Disappearance

The Manometric Method.—The manometric method of following the absorption of oxygen during the enzymic oxidation of a suitable substrate has been employed with advantage by several workers. The general technique is fully described by Dixon [11] Richter[4] first attempted an estimation of the enzyme from the rate of O_2 uptake with catechol as substrate, employing a preparation of the enzyme from potato. Graubard and Nelson worked out in detail conditions for an accurate estimation of the activity, and found *p*-cresol a better substrate on account of the lower and steadier O_2 uptake and observed that at low concentrations of enzyme, the slopes of the O_2 uptake–time curves were directly proportional to the enzyme concentration.

We have investigated the O_2 uptake during the oxidation of several substrates by the enzyme extract from *Dolichos lablab*, and have standardised conditions for obtaining an accurate measure of the enzyme. Tyrosine appealed as the direct substrate, but results with it were as discouraging as the bromination method. *p*-Cresol here, too, proved an excellent substrate with a rate of O_2 uptake, over a fairly wide range of enzyme concentrations, proportional to the enzyme.

We have employed the Warburg constant volume manometer with reaction vessels of approximately 15 c.c. total capacity, carrying one side bulb.

The total volume of the reaction mixture was always 2 c.c. and the temperature of the thermostat maintained at 30° C. Brodie's solution was used as the manometric liquid. Thus the readings of the manometer (difference in levels in *mm.*) are directly proportional to the O_2 uptake in *cmm.* and hence the direct readings in *mm.* have been taken in all cases for comparison —the absolute volume of O_2 utilised being calculated only in cases where that was specifically necessary or desirable. (The constants for the vessels varied from 1·210 to 1·212.) The manometer and attached vessels were shaken at 70–80 complete oscillations per minute, but, between 60–100, there was no appreciable difference in the rate of O_2 uptake with the shaking. With *p*-cresol as substrate, conditions of pH, substrate concentration, etc., were standardised for an accurate measure of the enzyme. The substrate concentration (between 0·25 mgm. and 5 *mgms.*) had practically no influence on the O_2 uptake rate. The pH employed was 6·2 (0·2 m. Na_2HPO_4—0·1 m. citric acid buffer). The conditions are :

$$\left.\begin{array}{ll} \text{p-Cresol} & = 1 \text{ mgm.} \\ \text{pH} & = 6\cdot 2 \\ \text{Enzyme + Water} & \end{array}\right\} \text{Total volume 2 c.c.}$$

A series of O_2 uptake curves for different enzyme concentrations are represented in Fig. 2.

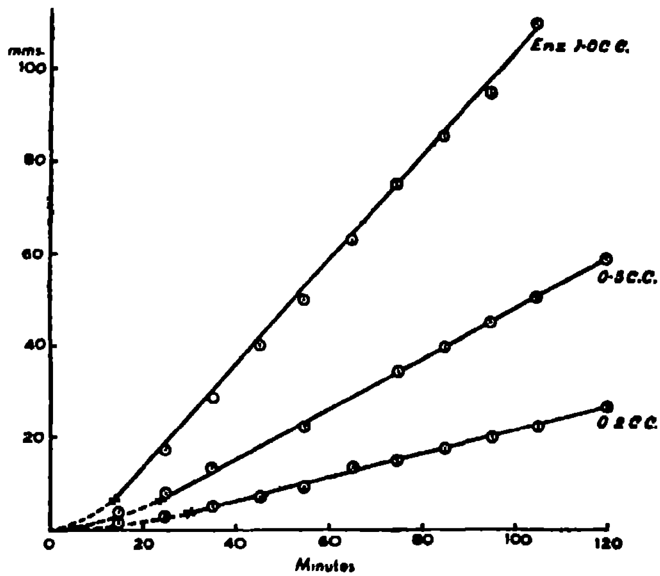


FIG. 2
p-Cresol : O_2 uptake

There is a short lag period in the initial stages, the duration of which varies with the concentration of the enzyme from 5–20 minutes and

afterwards the oxidation proceeds with practically constant rate of O_2 uptake till nearly the end, about 3 atoms of O_2 being absorbed per molecule of *p*-cresol oxidised. This steady rate could be noted from the slope of the curve or more directly, from the successive manometric readings themselves. The corrections due to changes in the temperature of the thermostat, or in the atmospheric pressure are, in general, negligible.

The limits of accuracy of the method were determined with one series, the enzyme amounts and corresponding O_2 uptake rates being given below : 0·7 c.c.–1·3 mm./min. : 0 8 c c –1 55 mm./min., 0·9 c.c.–1·85 mm./min., 1·00 c.c. –1 95 mm./min.

Thus results with ± 5% accuracy could be obtained easily. An arbitrary unit of the enzyme could be defined as that amount which promotes O_2 uptake (by *p*-cresol under standard conditions) of 10 mm /min. The number of units in relation to the total solids content of a certain preparation of the enzyme is a measure of its purity.

In a study of the nature of the enzyme tyrosinase, in view of recent work[6-8] on this group of enzymes, it is of great importance to examine the substrate specificity of the enzyme. The existence of a separate enzyme specifically attacking mono-hydroxy phenols has been questioned by many, who hold the oxidation of these to be merely a secondary phenomenon, the enzyme responsible being a di- or poly-hydroxy phenol oxidase. This view is disputed by others. Hence the importance of studying the behaviour of the enzyme towards suitable mono- and di-hydroxy phenolic substrates, at different stages of purification, cannot be overèmphasised

The Oxidation of Catechol.—The dihydroxy phenol, catechol, has been used widely as an experimental substrate in the study of phenolase activity (Richter,[4] Kubowitz,[6] Keilin and Mann[7]) The oxidation of catechol by tyrosinase which is generally rapid, is accompanied by an inactivation of the enzyme. This is brought about by the *O*-quinone which is formed during the reaction and efforts were directed to eliminate it from the sphere of the reaction. Richter succeeded in securing a fair degree of accuracy in measuring the activity of the enzyme by carrying out the reaction in the presence of aniline, which combines with *O*-quinone. Kubowitz, on the other hand, introduced a reducing system—thus making the function of catechol a " carrier " of oxygen—using hexose monophosphate with its corresponding apo-dehydrogenase and coenzyme. Other more easily accessible substances could be used as the substrates for " carrier " oxidation, *e g*., ascorbic acid and hydroquinone, both employed by Graubard[8] and potassium ferricyanide.

Of the substances so far examined, catechol is most easily and rapidly oxidised by the *Dolichos lablab* enzyme preparation. Next in order comes the other catechol derivative, dihydroxy phenyl alanine, commonly known as "dopa". The course of oxidation follows lines closely similar to that of the enzyme studied by Nelson and Adams.[9] The initial O_2 uptake is very high but tends to decline rapidly unless the proportion of the enzyme to the substrate is very high. There is no conceivable proportionality to the concentration of the enzyme—even during the first minute or two—except as an approximation. With lower concentrations of enzyme, the decline in rate of O_2 uptake is rapid and the O_2 uptake becomes negligible in the course of 10 to 15 minutes. This is presumably due to inactivation of the enzyme. The total O_2 uptake does not prove to be any complete, but corresponds to 2 atoms of O_2 per molecule catechol, when the enzyme concentration is very high. With the larger concentrations of the enzyme, the uptake of the first atom of oxygen is very rapid while the rate is lower during the uptake of the second atom. Neither is the total O_2 uptake during any stage of the oxidation proportional to the concentration of the enzyme. Thus the direct oxidation of catechol cannot, in any way, be employed as a method of measuring the enzyme. Fig. 3 gives the curves for the O_2 uptake by catechol with varying concentrations of the enzyme.

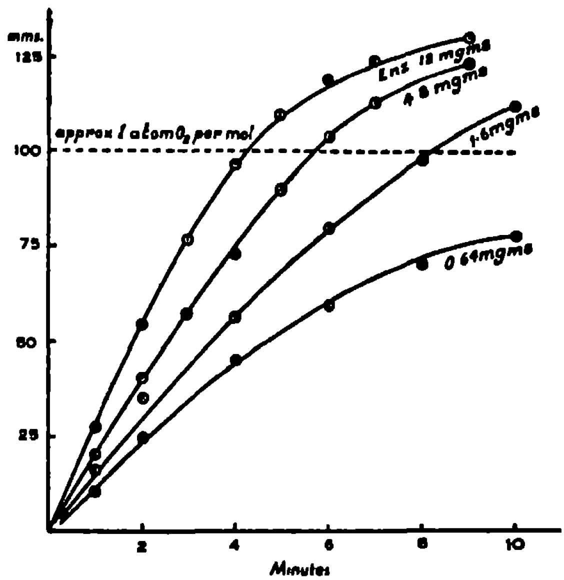


FIG. 3
O_2 Uptake by Catechol

It is thus clear that we are obliged to adopt the method of "carrier" oxidation with catechol as carrier, for measuring the catechol·oxidase activity of the enzyme preparation. Both hydroquinone and ascorbic acid are not directly oxidised by the enzyme, but the addition of a trace of catechol to the reaction mixture brings about this oxidation. Alternate oxidation of catechol by the enzyme and reduction of the quinone formed by these substances, is the mechanism that results in the O_2 uptake. With either ascorbic acid or hydroquinone as the (secondary) substrate, O_2 uptake is proportional to (1) a varying concentration of catechol for a given concentration of the enzyme, within the narrow limits of 0 005 to 0·025 mgm. in 2 c.c., (2) varying concentration of the enzyme for a given quantity of catechol, over a fairly wide range of enzyme concentrations. Curves representing the above results are given in Figs. 4–5. Most suitable conditions for a measurement of the enzyme activity are :

Hydroquinone or ascorbic acid	2 mgm.	Total volume 2 c.c.
Catechol	0 02 mgm.	
Buffer pH	6·2	
Water + enzyme		

An enzyme unit for the determination of catechol-oxidase activity similar to that previously described for *p*-cresol-oxidase, can be fixed, so

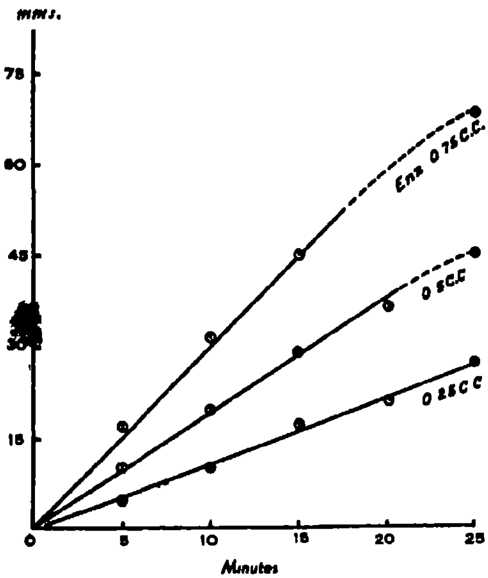


FIG. 4
Hydroquinone/Catechol

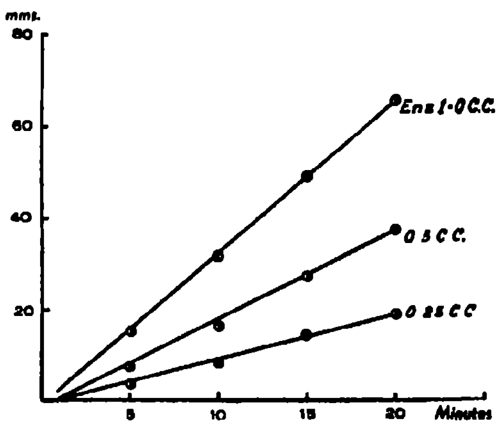


FIG. 5
Ascorbic acid/Catechol

that a comparison of the activities towards the two substrates at different stages of purification, could be made.

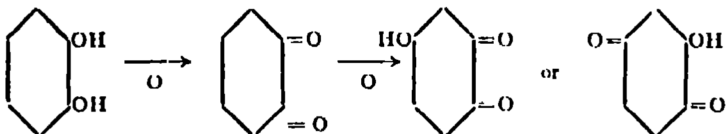
The total O_2 uptake when ascorbic acid or hydroquinone is oxidised, corresponds to 1 atom oxygen per molecule. The oxidation of ascorbic acid proceeds at very nearly twice the rate of that of hydroquinone, under identical conditions of catechol and enzyme concentration. Extreme care needs to be taken when ascorbic acid is employed as substrate, against traces of extraneous copper in the reaction mixture. Potassium ferrocyanide is also oxidised by the use of catechol as "carrier".

Products of Oxidation of Catechol: Iodimetric Method of Estimation.—The enzymic oxidation of catechol was noted to involve the uptake of 2 atoms of oxygen. *O*-quinone was suggested to be the product of oxidation and several facts were cited in the evidence for this view, till a conclusive confirmation came from Raper's[10] isolation of *O*-quinone derivatives from the reaction mixture Since the formation of *O*-quinone requires only one atom of O_2, the fate of the second atom taken up remained obscure. After a detailed study of the course of oxidation, Adams and Nelson[9] now suggest a scheme of oxidation which should result in the formation of a hydroxy quinone. They have developed a method for following the course of oxidation of cetechol, estimating the quinone formed at various stages, by its ability to liberate iodine from acidified potassium iodide. We have also studied this method with a few alterations in the procedure so as to suit a direct comparison of the results with the O_2 uptake.

In a 100 c.c. conical flask, are placed 12·5 c.c. of a solution of catechol containing 1 mgm. per c.c. and 3 0 c.c. buffer (0 4 M Na_2HPO_4–0·2 M citric acid, pH 6·2) and enzyme extract and water, to bring the final volume of the reaction mixture to 25 ml The flask is stoppered and shaken in a thermostat, maintained at 30° C. Soon after the addition of the enzyme, 2 c c. of the reaction mixture is removed from the flask and treated with 25 c.c. dilute 2N H_2SO_4. Subsequently 2 c.c. aliquots of the reaction mixture were removed at fixed intervals, treated with dilute H_2SO_4, and the quinone formed estimated iodimetrically. 10 c c. of 10% potassium iodide was added to the aliquots, the mixture kept in the dark for 15 minutes, and the liberated iodine titrated against N/100 thiosulphate.

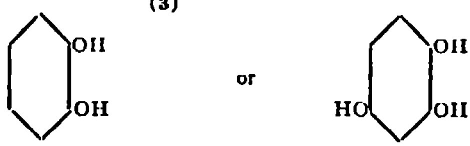
The course of formation of quinone bodies from catechol follows lines closely similar to that of the enzyme studied by Adams and Nelson. The conversion of catechol into the quinone is quick and quantitative only in presence of large concentrations of the enzyme. The complete conversion corresponds to an O_2 uptake equivalent to 1 atom O_2 per molecule catechol. But

the subsequent reaction which involves a further O_2 uptake observed in the manometer and which proceeds at a lower rate, yields products which do not give corresponding increases in the liberation of iodine. On the other hand, there is a steady decline in the value of iodine titre, which may be attributed to the instability of *O*-quinone under the experimental conditions. The iodine titre completely vanishes at the end of 3–4 hours. With lower enzyme concentrations, the oxidation to quinone is never complete but the iodine titre, after reaching a maximum value far lower than what should correspond to the complete formation of quinone, falls off. The experimental conditions were chosen so similar, as to have the iodine titre values and manometric readings directly correspond to each other



(1) (2) (3)

and (2) or (3) + $2HI \rightarrow I_2$ +



$$I_2 = 2Na_2S_2O_3.$$

i.e., 1 mM. catechol completely oxidised to quinone $\} = 2$ c.c. N. $Na_2S_2O_4$

i.e., 1 mgm. catechol ,, $= 1.82$ c.c. N/100 thiosulphate.

We have moreover applied the same method for following the course of oxidation of hydroquinone through catechol as "carrier", as also of *p*-cresol, "dopa", tyrosine and phenol. The method is applicable here also, and can be adopted as an alternative to every case where a measure of the enzyme is obtained by the O_2 uptake and quinone is a product of the oxidation. With phenol and *p*-cresol the initial induction period is indicated by the absence of any iodine liberation during that period.

Oxidation of other Substrates.—Phenol is easily oxidised by the enzyme, and the rate of O_2 uptake, after an induction period, remains steady and proportional to the concentration of the enzyme. The rate of the O_2 uptake is higher than with *p*-cresol, for the same concentration of enzyme. Tyrosine also exhibits an induction period, but the rate of O_2 uptake afterwards is neither steady for a long period nor proportional to enzyme concentration. "Dopa" is oxidised and the course of oxidation is very similar to that of catechol. It can also, similarly, serve as a "carrier" in the oxidation of ascorbic acid or hydroquinone. Among other substrates, the enzyme

oxidises *m*-cresol and pyrogallol, but *o*-cresol, vanillin, *p*-phenylene diamine and resorcinol are not oxidised.

Summary

As a preliminary to a purification and study of the nature of the enzyme, methods for a quantitative estimation of tyrosinase have been standardised. The O_2-uptake with mono- and di-hydroxy phenols has been studied in this connection. While the direct oxidation of phenol and *p*-cresol serves admirably as a measure of the enzyme, the oxidation of catechol fails to fulfil the conditions. To secure a steady rate of O_2-uptake proportional to enzyme concentration, the direct oxidation of catechol cannot be adopted but the oxidation of either ascorbic acid or hydroquinone (or ferricyanide) through the agency of catechol as " carrier ", fulfils the requirements. The formation of quinone bodies, the products of oxidation of various substrates by the enzyme, could be followed also iodimetrically.

The general substrate specificity of the enzyme suggests that it is not a " laccase " since the enzyme preparations have been found to be inert towards *p*-dihydroxy compounds. The fact that the enzyme preparations oxidise several mono- and di-hydric phenols, necessitates a deeper study of the influence of further purifications on substrate specificity.

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GLUTATHION IN ANÆMIAS

Its Variations in the Blood and Its Relation to the Erythrocyte Count and Hæmoglobin Content

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ANÆMIA signifies a blood condition in which the number of red cells, the total volume of cells and the quantity of hæmoglobin are decreased considerably owing to an imbalance between processes of destruction and replacement (Wiggers). Most of the investigations on anæmias are concerned with the formed elements of the blood. The biochemical investigations of the blood in anæmias are confined to the study of the physical and chemical characters and fate of the blood pigment. Glutathion is one of the important constituents of the erythrocyte, susceptible to variations in blood diseases.

The blood glutathion is entirely confined to the erythrocytes; the variations in its values in most of the pathological conditions, even in cystinuria, are within normal limits; in liver diseases the variations are wide; and in anæmias and some cyanotic conditions the ratio of glutathion to erythrocytes is increased (Platt, 1931). Rabbits rendered anæmic after successive bleedings show an increase in blood glutathion per unit volume of red cells associated with the decrease in cell volume (Litarczek *et al.*, 1931) Generally when cell volume is low in anæmic cases, the whole blood reduced glutathion is reduced below normal mean and the figure per 100 c.c. cells is high; the opposite is true in polycythemic cases (Bowman, 1934).

In rabbits rendered anæmic by bleeding or by administration of phenyl hydrazine, the glutathion content of the whole blood is decreased and that of the erythrocyte is increased (Besozzi and Zanini, 1935) Corpuscular glutathion is increased in anæmias but decreased in polycythemias; the increase in post-hæmorrhagic and acute infectious anæmias being not well marked (Dogliotti and Castellani, 1935).

In rats suffering from experimental nutritional anæmia the total glutathion of the erythrocyte is decreased, there being a marked shift towards the oxidised form. In pigs under similar experimental conditions, there is an increase in both forms of glutathion in the erythrocyte as the anæmia becomes

severe. This difference is possibly due to the appearance of reducing substances other than glutathion in the blood of pigs or other animals during anæmia. The blood glutathion concentrations return to normal with the feeding of copper and iron (Schultze and Elvehjem, 1936).

The observations of several authors on the variation of glutathion in blood, lead one to infer that the blood glutathion, the erythrocyte count, and cell volume, are all interrelated. The occurrence of high corpuscular glutathion in pernicious anæmia and myelogenous leukæmia when the erythrocytes are megalocytic and hyperchromic (Schultz, 1939) justifies such an inference. But the observations of Kandel and LeRoy (1939) that the variations of blood glutathion cannot be correlated with either erythrocyte and leukocyte counts or hæmoglobin concentration or cell volume indicate that there are no grounds for such an inference. We have examined the blood of anæmic patients with respect to the glutathion content, erythrocyte count and hæmoglobin concentration with a view to arriving at any correlation that may exist.

The results of the investigation are embodied in Tables I and II. The blood counts range from 0 51 to 5 03 millions per mm.³, hæmoglobin from 2·3 to 14 8 gm. per 100 c.c. of blood and the colour index from 0·59 to 2 29. The average figures for reduced and oxidised glutathion are 21·39 and 4·60 mgm. per 100 c.c. blood. Gabbe's quotient and the modified Gabbe's quotient range from 4·1 to 27·7 and 1·9 to 9·5 with averages at 10·6 and 4 6 respectively.

Table III is an analysis of Tables I and II and gives the average values of the erythrocyte count, the reduced and oxidised glutathion content, the Gabbe's quotient for subjects suffering from various diseases causing anæmia. For ready reference the corresponding figures for healthy subjects are included in the same table. The average figure for Gabbe's quotient indicates a high corpuscular glutathion content in anæmia. It is the highest for ankylostome anæmias.

Fig. 1 shows that the variations of Gabbe's quotient with the erythrocyte count tend to be high with low cell counts. Fig. 2 shows that the variations of the modified Gabbe's quotient with the erythrocyte count tend to be confined to a narrow limit.

The results of the present study indicate that in human anæmias the corpuscular glutathion is increased more than normal as is evidenced by a high Gabbe's quotient. Though the whole blood glutathion content is within the observed limits for healthy individuals, the average values for reduced and oxidised glutathion show a slight decrease.

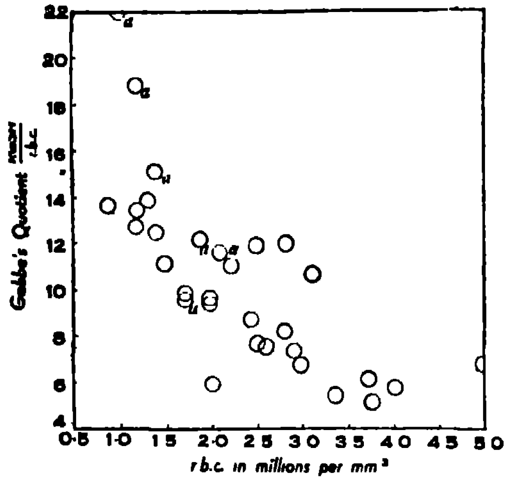

FIG. 1

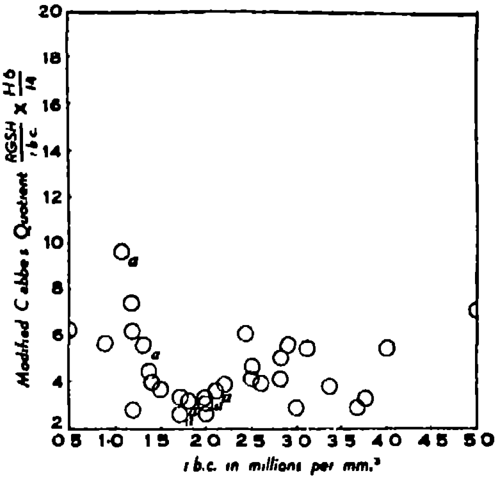

FIG. 2

a = ankylostome anæmia

According to Varela (1931) the blood glutathion in anæmias is decreased on account of diminution in red cells. If that be to the same extent, Gabbe's quotient should have either a constant value or its average value in anæmias should not differ much from the normal. The average value in the present investigation is 10·6 as against about 6 for healthy people. It may therefore be inferred that in anæmias the fall in the blood glutathion is compensated to a certain extent by a rise in corpuscular glutathion. Fig 3 indicates that

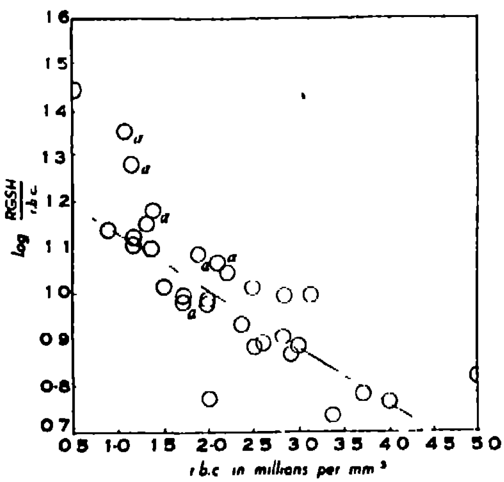

FIG. 3

TABLE I

First Series

Sl No	Case	Age, Sex	Diet	R b c × 10^6 per mm ^{3}	Hb in gm %	Colour Index	Glutathion in mgm in 100 c c blood			Gabbe's Quotient $\frac{GSH}{R.b\,c}$	$\frac{GSH}{R\,b\,c} \times \frac{Hb}{14}$	Clinical Notes
							Total	Reduced	Oxidised			
1	N	14 m	v	3 28	9 8	1 08	21 50	17 80	3 70	5 4	3·8	General anasarca c. anæmia
*2	M	30 f	nv	3 13	6·5	0 75	46 20	12·70	33 50	4 1	1 9	Anæmia and diarrhœa
3	V	30 f	v	2 43	9 9	1 47	29 45	20 90	8 55	8 6	6 0	Helmenthiasis
4	T	19 f	nv	1·65	3 7	0 81	20 86	15·34	5 52	9 3	2 4	Ankylostomiasis
5	R	30 f	v	2 53	8·5	1 21	25 48	18 41	7·07	7·5	4·4	Syphilitic anæmia
6	P	50 m	nv	1 25	5 5	1 58	25 77	17 18	8·59	13 7	5·5	Pernicious anæmia
7	M	20 m	,,	2 14	5 1	0 86	25 15	23 34	1·81	10·9	3 9	Tape worm infection
8	NG	55 m	,,	3 38	5 5	0 59	30 67	22 39	8 28	6·6	2 6	Secondary anæmia c. cirrhosis of liver and ascitis
9	SB	30 f	v	2 83	7 4	0 94	35·58	27·65	7·93	9 8	4 9	Chronic diarrhœa, amœbiasis
10	G	31 f	nv	3 11	7 9	0 91	32 52	30 06	2 46	9 7	5 4	Chronic diarrhœa, secondary anæmia
11	A	16 f	,,	4 03	13·3	1 19	31 29	23·31	7 98	5 8	5 5	Chronic malaria
12	V	50 f	v	5 03	14 8	1 06	36 86	33 13	3·73	6 6	7 0	Senile cataract; recovered anæmia
13	T	50 m	,,	2 04	3 4	0 60	24 54	19 63	4·91	9 6	2 4	After hæmorrhoids
14	N	30 m	nv	2 95	5 1	0 62	29·45	22·70	6 75	7 6	2 7	
15	GG	42 m	,,	1·95	4 7	0 87	20 86	18 41	2 45	9 4	3 2	Secondary anæmia
16	HG	50 m	,,	1 18	7 5	2 29	20 25	15 64	4 61	13 4	7 3	Chronic malaria

17	SG	30 m	,,	0·51	3·1	2·19	15·34	14·42	0·92	27·7	6·1	Nutritional anæmia
18	T	26 m	,,	0·94	5·2	1·99	20·25	12·88	7·37	13·6	5·6	Syphilitic anæmia
19	N	20 m	,,	2·05	4·3	0·76	25·77	23·62	2·15	11·5	3·4	Ankylostomiasis
20	R	15 m	v	2·91	10·5	1·30	25·15	21·47	3·68	7·3	5·5	Chronic malaria
*21	L	20 m	nv	4·43	13·0	1·05	45·39	25·76	19·63	5·8	5·4	Sprue
22	K	28 m	,,	1·68	4·8	0·98	17·18	15·34	1·84	9·6	3·3	Syphilitic anæmia
23	N	20 f	v	3·66	6·4	0·63	27·61	22·09	5·52	6·0	2·8	Secondary anæmia chlorotic
24	S	30 f	,,	1·48	4·5	1·12	17·18	16·56	0·62	11·2	3·7	Syphilitic anæmia
25	M	42 m	nv	1·22	3·0	1·11	16·56	15·34	1·22	12·5	2·6	Pulmonary tuberculosis
26	S	40 m	,,	2·49	4·9	0·71	36·20	29·45	6·75	11·8	4·1	After hæmorrhoids

Table II

Second Series

Sl. No.	Case	Age, Sex	Diet	R.b c × 10^6 per mm^3	Hb in gm %	Colour Index	Glutathion in mgms in 100 c c. blood			Gabbe's Quotient $\frac{GSH}{R\ b\ c}$	$\frac{GSH}{R\ b\ c} \times \frac{Hb}{14}$	Clinical Notes
							Total	Reduced	Oxidised			
27	M	30 m	nv	1·83	4 9	0 96	20·62	17 66	2·96	9·7	3 4	Nutritional anæmia
28	C	40 m	,,	1 88	7 0	1 34	23 93	22 39	1·54	12 0	6 0	Ankylostomiasis
29	N	40 m	,,	2·03	7 6	1 36	13·49	11·96	1·53	5 9	3·3	Ankylostomiasis and malaria
30	P	35 m	v	1 86	7·1	1 38		20·86		11 2		
31	A	60 m	nv	3 76	9 3	0 89	29 45	18 40	11 05	4 9	3 3	Senile cataract, recovered anæmia
32	V	23 m	v	4 51	10 2	0 81		34 66		7 7		Chronic malaria
33	M	25 m	,,	1·23	2 3	0 67	22 70	22 70	Trace	18 5	6 1	Ankylostomiasis
34	V	23 m	,,	1 43	4 2	1 06	30 37	21·17	9 2	14 8	4·4	Ankylostomiasis
35	N	35 m	nv	4 38	12 1	0 99	57 53	48 90	8 63	11 2	9 6	Healthy
36	V	30 m	,,		4·7		28 22	26 08	2 14			Chronic malaria
37	X	23 m	,,	1 09	6 1	2 01	26 58	23 93	2 65	22 0	9 5	Ankylostomiasis
38	M	35 m	,,	1 40	4·5	1 16	20 86	17 18	3 68	12 3	3 9	Chronic malaria
39	N	30 m	,,	2·63	7·0	0 96	25 77	20 24	5 53	7 7	3 9	Nutritional anæmia
40	K	25 m	,,	2 80	7 0	0 90	24 52	22 39	2 13	8 0	4 0	Ankylostomiasis, chronic malaria

m = male, f = female, v = vegetarian, nv = non-vegetarian.

TABLE III

Averages in the Several Anæmias

No. of cases examined	Cause of Anæmia	R b c. $\times 10^6$ per mm ^{3}	Glutathion in mgm. in 100 c c blood			Gabbe's Quotient	Modified Gabbe's Quotient
			Total	Reduced	Oxidised		
6	Ankylostomiasis	2 2	25 04	21·53	3 51	14·7	5·3
5	Chronic malaria	2 2	25 15	20 74	4 41	9·7	5·6
2	Chronic malaria and Ankylostomiasis	2 4	19·01	17 18	1 83	6 9	3·6
22	All other causes	1 9	27 02	20 86	6 16	10 5	4·3
* Average for the above 35 cases		2·0	25 99	21 39	4 60	10 6	4 6
Average for 26 healthy cases having senile cataract			33·45	25 76	6 72	6·1	5 1
Average for 30 young healthy adults (Woodward and Fry)				34 00		6 8	6 6

* Forty cases were examined but the average is given for only 35 Five cases have not been taken for calculation either because all the factors were not determined or they gave abnormal values as in Nos 2 and 21

the increase in corpuscular glutathion in the present series of anæmic subjects is such that the logarithm of Gabbe's quotient is inversely proportional, in a majority of cases, to the erythrocyte count. But Kandel and LeRoy (1939) are of opinion that the variations of glutathion follow crudely the changes in the number of formed elements of the blood; the variations in patients with hæmatologic diseases are not found statistically significant from similar variations in patients without such diseases.

Bach and Bach (1931) have found an increase in the glutathion and catalase content of the erythrocyte in pernicious anæmia and not in secondary anæmias. They believe that conditions which lead to an increase of oxygen consumption increase the glutathion and catalase content of the erythrocyte but not conditions that give rise to megalocytosis and hyperchromy. That the increase in the oxygen consumption of anæmic blood is a function of the erythrocytes alone and that it is proportional to the reticulocyte count of the blood has been observed by Litarczek and his co-workers (1935). It follows as a corollary that the increase in the corpuscular glutathion is associated with reticulocyte count. This partly explains the blood glutathion findings of Dogliotti and Castellani (1935) in post-hæmorrhagic and acute infectious anæmias; because in acute post-hæmorrhagic anæmia, the reticulocytosis that is induced by the loss of blood rapidly decreases and disappears ; and in chronic post-hæmorrhagic anæmia the reticulocytes are scanty (Piney, 1939); and since the depressing effect of infection on hæmopoietic activity of the bone marrow is indicated by the low reticulocyte count found during the febrile stage of any disease (Tice) it is possible that the infection suppresses the hæmopoietic activity in acute infectious anæmias. It is therefore to be expected that the increase in corpuscular glutathion in anæmias is dependent on the nature of the anæmia.

Litarczek *et al* (1931) have found that in bled rabbits the content of reduced glutathion in the erythrocyte and the dissociation constant, *i*/K of hæmoglobin show a parallel rise and in clinical cases the increased values for corpuscular glutathion and the *i*/K run concurrently with the lowering of the oxygen capacity of the blood According to Pickard and Marsden (1934) there exists no quantitative relationship between the hæmoglobin and glutathion in the blood, the latter is lowered when there is a sudden loss of blood and restored to the normal before hæmoglobin and the number of erythrocytes; the quantity of glutathion does not follow variations of cell count or cell volume though it forms part of the erythrocyte. But Besozzi and Zanini (1935) think that the increase in corpuscular glutathion is a compensatory reaction of the organism against loss of oxidative power in anæmias. Woodward and Fry (1932) also believe that there may exist a compensatory

relationship between the amount of hæmoglobin and the glutathion of the blood. It is possibly on account of this that the variations in modified Gabbe's quotient are confined between narrow limits even when the erythrocyte counts vary very widely.

Experimental

40 Anæmic patients (10 female and 30 male) between 14 and 55 years of age undergoing treatment in the Krishnarajendra Hospital were the subjects of this investigation.

Glutathion determinations, hæmoglobin estimations, and erythrocyte counts were all made on the same sample of blood drawn from the median cubital vein. The procedure adopted was the same as in Glutathion in ocular diseases (Srikantia *et al.*, 1940).

The first series differed from the second in that oxalated blood was not used in the second series. The blood for the several estimations was pipetted out immediately after withdrawing it, into several vessels kept ready near the bed-side of the patients and brought to the laboratory for completing the estimations. The manipulation of pipetting out the blood from the receptacle to which it was transferred from the syringe, scarcely took two minutes with practice.

Colour index was calculated by multiplying the hæmoglobin concentration per million cells by 0 36, the reciprocal of the standard. This factor, 0·36, is the same for both sexes, and is found to be approximately the same in the several standards.

Summary

Glutathion determinations, hæmoglobin estimations and erythrocyte counts have been made with the venous blood of each of 40 anæmic patients; Gabbe's and modified Gabbe's quotients calculated

The values range from (i) 11·96 to 48 90 mgm for reduced glutathion; (ii) traces to 19 63 mgm. for oxidised glutathion in 100 c.c. of blood; (iii) 2 3 to 14 8 gm. for 100 c.c. blood for hæmoglobin, and (iv) 0 51 to 5 03 millions per mm.^{3} for the erythrocyte count. Gabbe's quotient tends to be high with low counts and appears to follow an exponential curve. Modified Gabbe's quotient tends to oscillate between narrow limits suggesting the possibility of an interrelationship between the hæmoglobin and the glutathion in the blood.

There is a marked increase in the corpuscular glutathion in anæmias generally and in ankylostome anæmias the increase is higher.

One of us (T. P. R.) desires to record here his grateful thanks to the University of Mysore for the grant of a research scholarship for carrying out this investigation.

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* References from abstracts and not from the original

Errata

Vol. XI, No 6, Section B, June 1940—

In page 267, Table I, 1st line, *for* " 85 ", *read* " 8·5 "
In page 267, Table I, 3rd line, *for* " 54·2 ", *read* " 54 7 ".
In page 267, Table II, 3rd line, *for* " 37 8 ", *read* " 37·7 ".

THE EFFECT OF ETHYLENE AND SULPHUR DIOXIDE ON THE FRUITS OF *MANGIFERA INDICA*

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Introduction

THE problem suggested itself to the senior author when, in 1936, he was called as an expert witness in a law court at Benares to give evidence on the damage caused to a mango orchard by fumes from brick-kilns. In order to consider the damages caused by fumes from brick-kilns it is important to know the constitution of the gas. The important constituents of the blast furnace gas are as follows :

TABLE I

	%
Carbon dioxide	8·5
Carbon monoxide	27·1
Nitrogen	54·2
Hydrogen	5·4
Ethylene ..	4·2
Sulphurdioxide ..	0·1

The composition of unoxidised coal gas is as follows :

TABLE II

	%
Hydrogen	36·1
Carbon monoxide	6·8
Marsh gas	37·8
Ethylene	16·4
Nitrogen	2·9
Sulphur dioxide and other gases	0·1

Thus, the gas emanating from brick-kilns would have a composition greatly varying between the limits of the two tables given above. Of the constituents, carbon monoxide, marsh gas, ethylene, and sulphur dioxide are highly injurious. It was, therefore, planned to work out the effect of these gases individually on various fruits. The effect of ethylene and sulphur dioxide on the fruit of *Mangifera indica* have been reported in this paper.

The effect of ethylene on fruits has been studied by many workers. Regeimbal, Vacha and Harvey[10] showed that respiration of bananas doubled or even trebled in ethylene (1 part of ethylene in 1,000 parts of air). Denny[3] found that the concentrations of this gas such as 1 : 1,000, 1 : 10,000, 1 : 100,000, and even 1 : 1,000,000 increased the respiration of green lemons. Allen,[1] working with apples, found that after ethylene treatments (1 · 1,000), lasting for 4–10 days, the treated fruits were softer and yellower than the non-treated ones. Hibbard[7] reported that the ripening of tomatoes and bananas was accelerated by ethylene treatment. Similar results have also been obtained by Kohman[8] and Wolfe.[12] The work of Harvey[5] indicates that, in sweet peas, ethylene (0 0002%) causes simpler compounds to increase at the expense of the more complex ones Hartshorn[6] and, more recently, Gane[4] confirm that the ripening of bananas is hastened by ethylene; moreover, evidence is obtained by the latter author, that ethylene is normally formed in bananas as a product of metabolism.

That sulphur dioxide is poisonous to vegetable life has been shown by Sconard[11] who investigated the effects of this gas on vegetation and found that it was injurious to plants. Zimmerman and Crocker[13] also concluded that injury resulted in tomatoes, peaches, roses and various other plants by 1–4 hour treatment with sulphur dioxide in such low concentrations as 3–8 parts of this gas in a million parts of air.

Recently, Das Gupta and Verma[2] made a preliminary survey of the 'black tip' disease in Lucknow, and found that the disease occurred in the mangoes growing in the vicinity of brick-kilns.

Materials and Methods

Healthy fruits of various ages were gathered locally from a single tree, washed with a solution of potassium permanganate and kept for some time. They were then examined for respiration, sugar and acid contents, and for pH.

Preparation of Ethylene and Sulphur Dioxide.—Ethylene used in this work was prepared in the laboratory in the following way Phosphoric acid and pieces of pumice were heated in a flask to nearly 230° C.; absolute

alcohol was dropped into it and the gas evolved was collected by displacement of water after the alcohol vapour was separated by condensation.

Sulphur dioxide was obtained by heating copper filings with concentrated sulphuric acid and collected in a flask by the displacement of air. The gas, thus stored, could be made into such concentrations as desired.

Analysis of CO_2.—Respiration measurements were made by the continuous current method with the aid of a Blackman Air Commutator using Pettenkofer tubes filled with barium hydroxide solution of definite strength. The alkali was titrated, after the respiratory current was drawn through the tubes, at fixed intervals of time. This interval was three hours throughout the work. In all cases two sets of experiments were conducted—one for control and the other for experimentation.

Sugar Analysis—For the analysis of sugar-content, the skin and seeds were removed and only the pulp was utilized. A known weight of the pulp was boiled, made into a paste and the water in which it was boiled was added to it. The tannins were precipitated by lead acetate and the excess of lead was removed by H_2S. The liquid was finally boiled to drive off the dissolved hydrogen sulphide and titrated against Pavy's solution.

Acid Determinations.—For acid determination, a known weight of the pulp was crushed with a little distilled water and centrifuged. A part of the liquid was used for pH determination and the remaining part for the total acid estimation. The former was found out by means of an electric potentiometer. For the latter a quantity of the solution was added to a known amount of dilute $\left(\text{approximately } \frac{N}{100}\right)$ solution of sodium hydroxide and this was titrated against standard oxalic acid $\left(\frac{N}{100}\right)$ The same quantity of sodium hydroxide was titrated directly with oxalic acid without the solution being added. The difference between the two gave the amount of acid present.

Experimental Results

Experiment I.—In this experiment mangoes of about 30 days old (from the same branch) were selected and ethylene-air mixture (1 : 1,000 dilution) was admitted after nine hours of respiration in the air. After 27 hours of ethylene treatment, the respiratory rate suddenly went up and then remained wavy (Fig. 1). After the experiment, the fruits were superficially examined but no significant difference was found between the control and the ethylene-treated fruits.

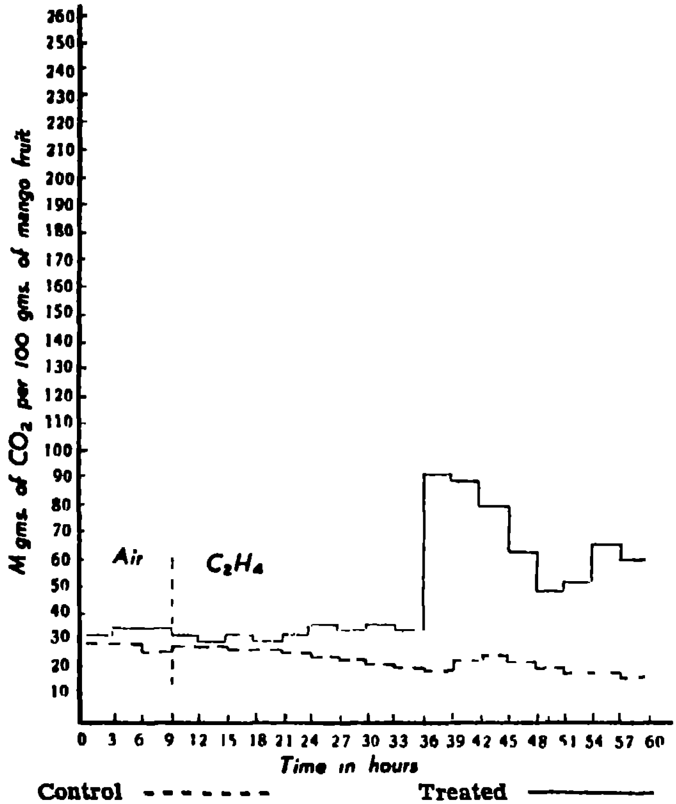


FIG. 1

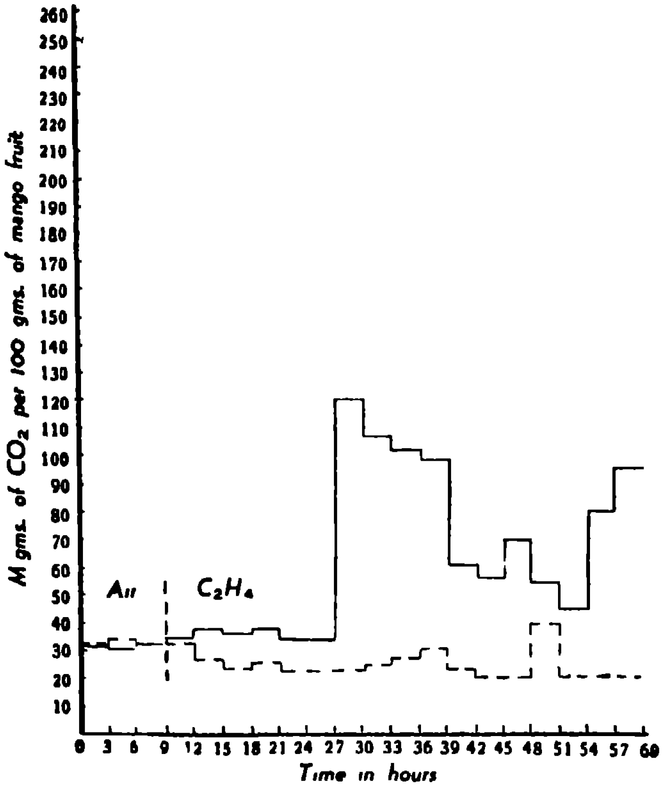
The sugar and acid contents of the fruits are given in the following table :

TABLE III

	Before Experiment	After Experiment	
		Ethylene Set	Control Set
Total acid	1 gm = 6 7 c c NaOH (N/100)	1 gm. – 6 68 c c. NaOH	1 gm. = 6·81 c.c. NaOH
pH	4·9	4 8	4 8
Monosaccharides .	1·32%	1 81%	1·28%
Disaccharides	1·1%	1·28%	1·17%

Experiment II.—The fruits selected were about 40 days old. Ethylene used was of the concentration of 1 : 1,000; after 18 hours of administration

of ethylene there was an enormous rise in the respiration rate (Fig. 2). Superficially there was not much difference in the fruits after the experiment.



Control - - - - - - -   Treated ————

FIG. 2

The sugar and acid values were as follows :

TABLE IV

	Before Experiment	After Experiment	
		Ethylene Set	Control Set
Total acid ..	1 gm = 8 1 c c NaOH	1 gm = 8 6 c c NaOH	1 gm = 8 07 c c NaOH
pH	4 28	4 3	4·19
Monosaccharides	1·4%	2·03%	1·86%
Disaccharides	1·2%	2·37%	1·69%

Experiment III.—Mangoes selected were about 50 days old and 1 : 1,000 ethylene-air mixture was used. There was a very great rise in respiratory rate after about 21 hours (Fig. 3). Examination of the fruits after the

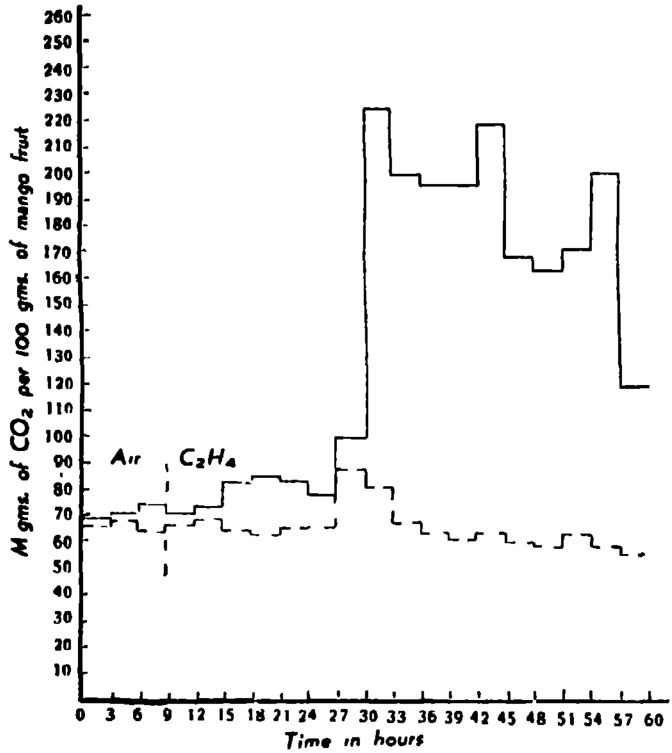


FIG. 3

experiment showed that the mesocarp had become pulpy, more so towards the distal end, and the skin (epicarp) had become loose from the pulp in the ethylene-treated fruits, while the control showed no change. Sugar and acid values were as follows :

TABLE V

	Before Experiment	After Experiment	
		Ethylene Set	Control Set
Total acid	1 gm. = 9·04 c.c. NaOH	1 gm. = 9·3 c.c. NaOH	1 gm. = 9·5 c.c NaOH
*p*H	4·04	4·2	4·1
Monosaccharides ..	2·6%	3·5%	2·82%
Disaccharides	1·9%	3·21%	2·01%

Experiment IV.—Mangoes selected were 60 days old and 1 : 1,000 dilution of ethylene was administered (Fig. 4). There was an irregular rise of the

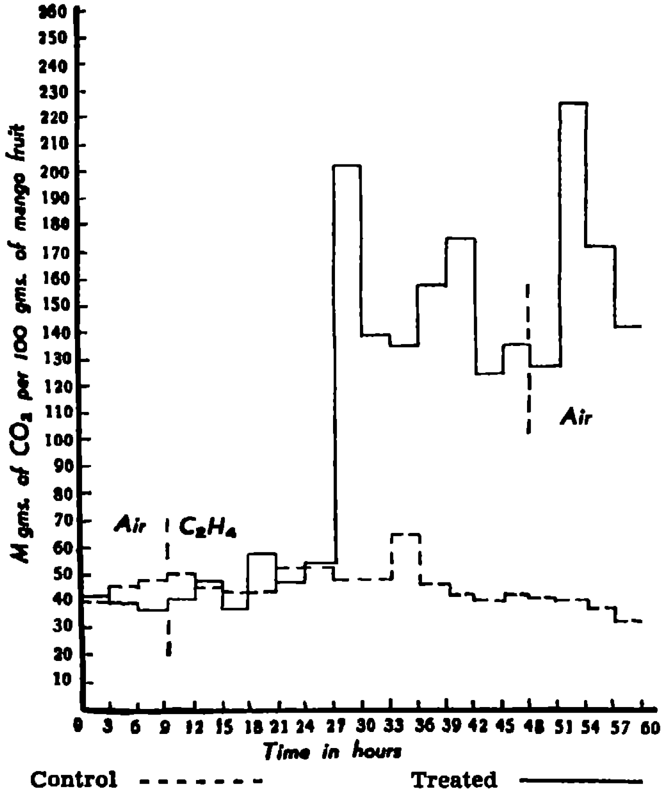


FIG. 4

respiratory rate in the first eighteen hours after which respiratory rate went up as in the preceding experiments. After the experiment it was found that the treated fruit had become absolutely pulpy and the green colour yellowish especially in the lower half ; internally also the mesocarp had become yellowish. Sugar and acid contents were as follows:

TABLE VI

	Before Experiment	After Experiment	
		Ethylene Set	Control Set
Total acid ..	1 gm. = 10·01 c c NaOH	1 gm = 9·25 c c NaOH	1 gm = 10·8 c.c NaOH
Monosaccharides ..	3·6%	4·8%	3·6%
Disaccharides	3·1%	6·2%	4·1%

Experiment V.—Here the mangoes about 70 days old were used. Fig. 5 gives the respiratory activity. Within 9 hours of admixture of ethylene, the

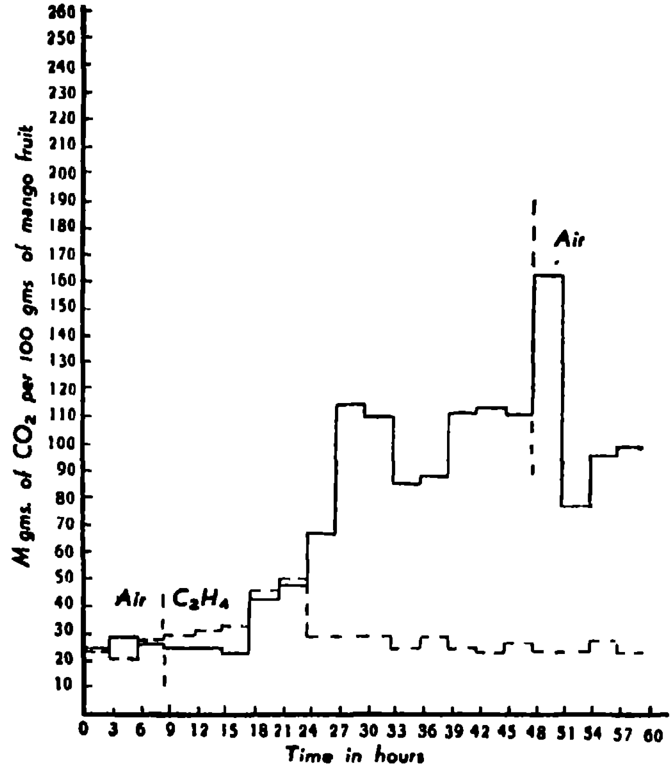


FIG 5

rate of respiration started to go up. The peak was reached after 18 hours of treatment with ethylene. When air was reintroduced, there was a slight after-effect and the respiration then fell off. The sugar and acid contents were as follows :

TABLE VII

	Before Experiment	After Experiment	
		Ethylene Set	Control Set
Total acid .. .	1 gm = 10·7 c.c. NaOH	1 gm = 11·2 c c. NaOH	1 gm. = 9·1c.c. NaOH
Monosaccharides .	3·6%	7·1%	4·3%
Disaccharides . .	6·8 %	7·23%	7·01%

Experiment VI.—In this experiment 60–70 days old mangoes were used with their skin peeled off. The initial rate of respiration was very high and this gradually came down and then again rose to a high level when ethylene (1 : 1,000) was given (Fig. 6). The treated fruit after the experiment was quite pulpy and the mesocarp yellowish.

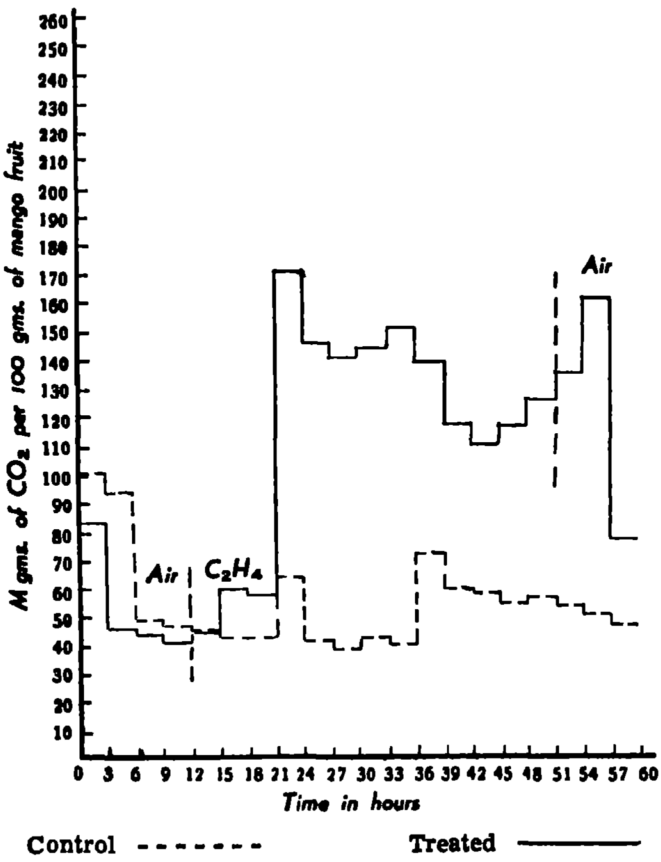


FIG. 6

Experiment VII.—Two mangoes of 80–90 days old were selected. After 12 hours of air respiration, coal gas-air mixture (1 : 4 dilution) was introduced in one chamber. The rate of respiration of treated fruit went up appreciably (Fig. 7) after 18 hours and still higher subsequently. After 63

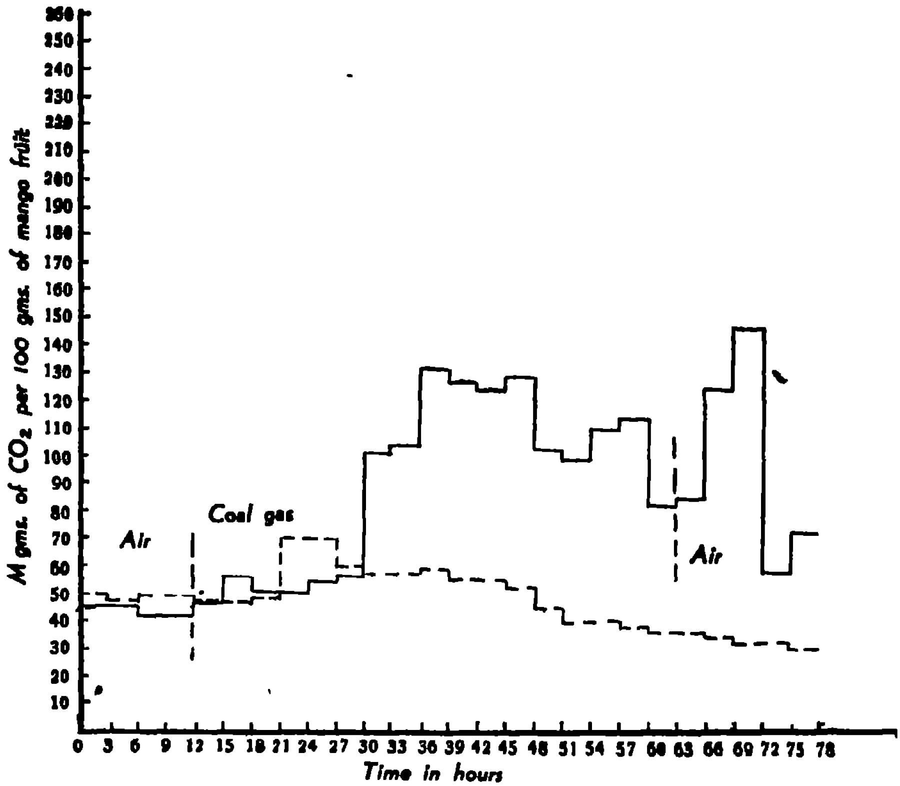


FIG. 7

hours the coal gas-air mixture was replaced by air and the subsequent respiratory rate went up and then came down. The condition of the fruits before and after the experiment was as follows :

TABLE VIII

Before Experiment		After Experiment	
		Coal-gas Set	Control Set
External condition	Hard	Soft	Hard
Skin colour . ..	Green	Green with brown yellowish patches	Light green
Internal condition ..	Greenish and hard mesocarp	Mesocarp soft and yellow, epicarp loose from mesocarp	Mesocarp hard, epicarp slightly loose from mesocarp

Experiment VIII.—Mangoes selected were 80–90 days old and sulphur dioxide-air mixture (1 : 1,000) was administered. The respiratory rate of the treated fruit rose very high after 18 hours of administration of the gas (Fig. 8). The treated fruit after the experiment was pulpy and its green

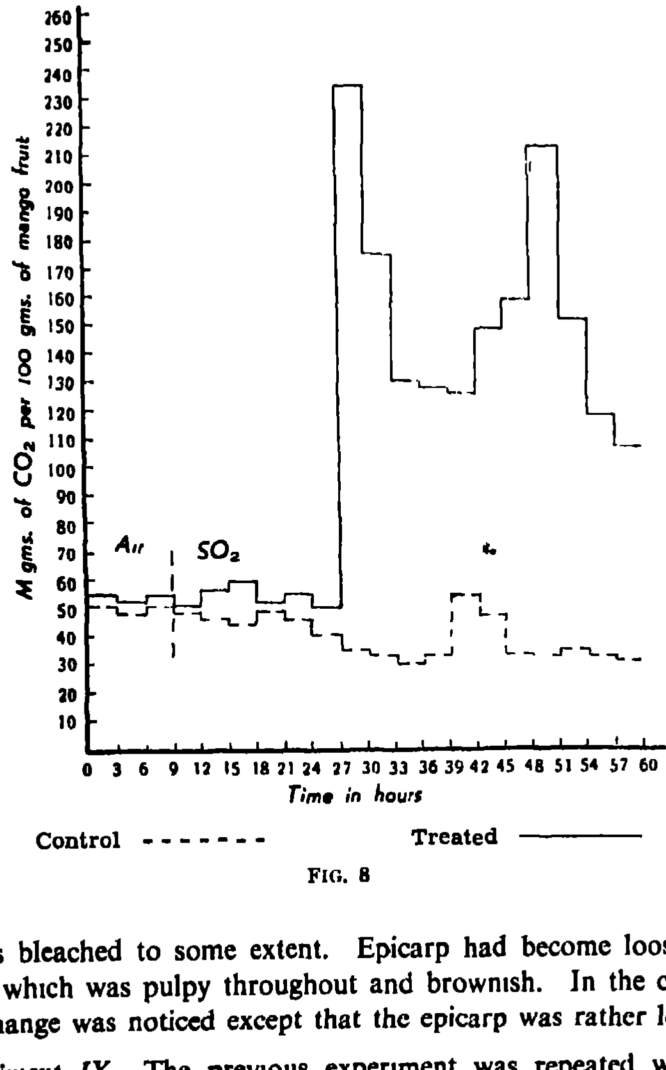


FIG. 8

colour was bleached to some extent. Epicarp had become loose from the mesocarp, which was pulpy throughout and brownish. In the control fruit no such change was noticed except that the epicarp was rather loose.

Experiment IX.—The previous experiment was repeated with 90–100 days old fruits. There was a steady rise in the respiratory rate at first, but after 15 hours the rate suddenly accelarated (Fig. 9). The lower half of the

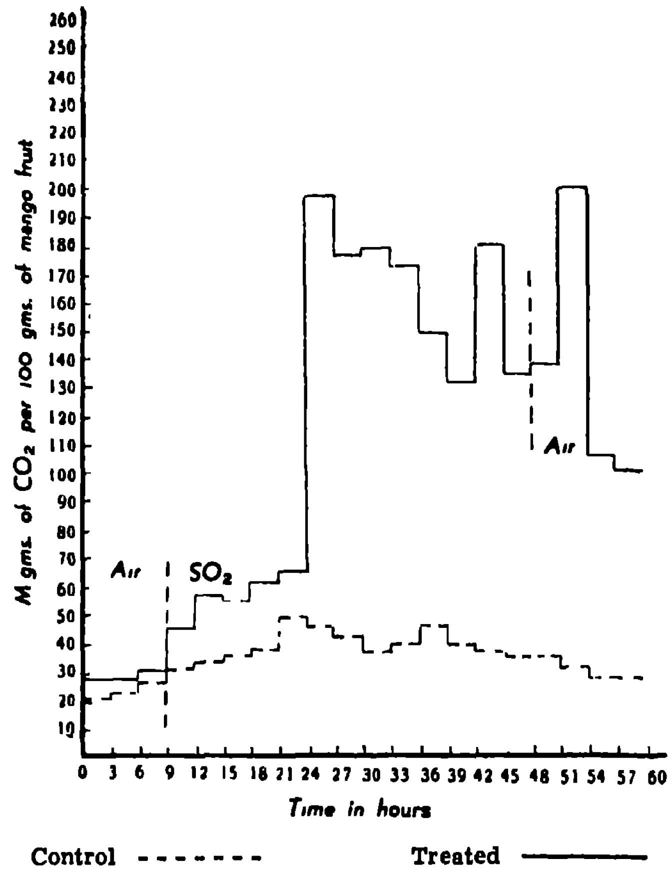
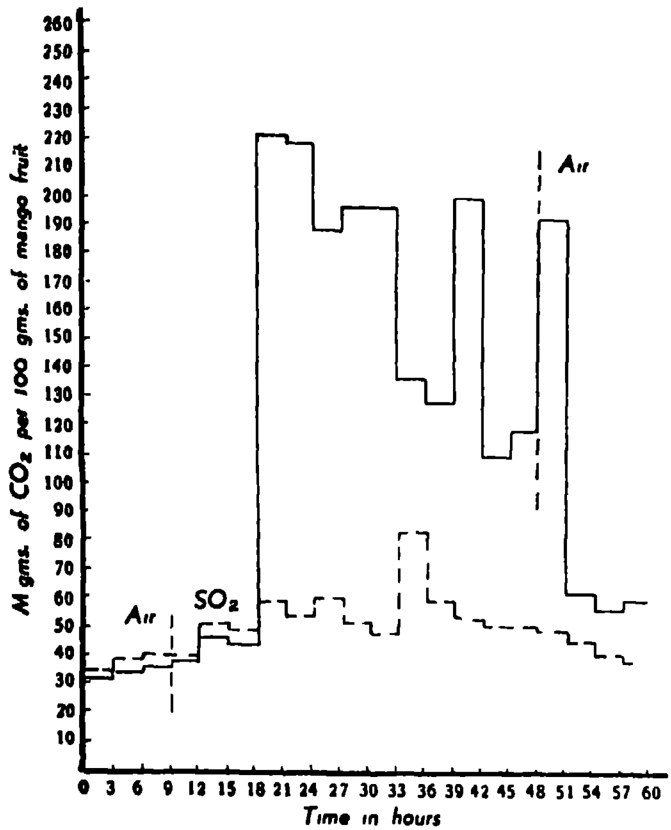


FIG. 9

mesocarp of the treated fruit was very much disorganized, very pulpy, and brownish in colour; it smelt foul like rotten fruit.

Experiment X—The same procedure as in Experiment IX was adopted except that the skin of the mangoes was peeled off before experimentation. There was a sudden, high rise in the respiratory rate of the treated fruit the lower half of which, after the experiment, was very much disorganized; the upper half was pulpy, the inner pulp having yellowish colour (Fig. 10).



Control - - - - - - - Treated ————

FIG. 10

Experiment XI.—Mangoes were about 50 days old to which ethylene (0 1%)-sulphur dioxide (0·01%)-air mixture was administered. There was a sudden rise in the respiratory rate of the treated fruit at the end of 12–13 hours (Fig. 11). The fruit had become soft and the colour yellowish-green

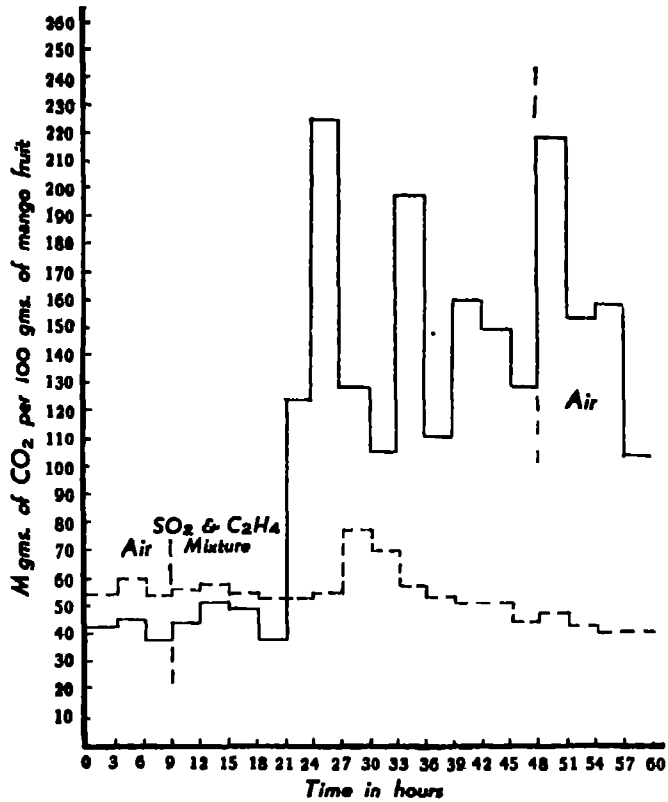


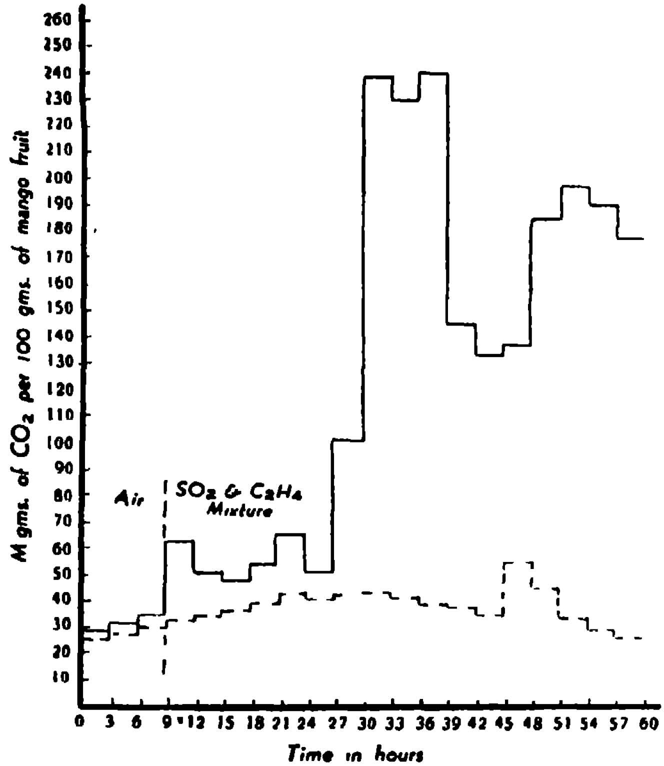
FIG. 11

after the experiment; endocarp was hard, mesocarp very much disorganized in the lower half and the colour of the pulp was whitish-yellow in the upper half and brownish in the lower. Sugar and acid values were as follows :

TABLE IX

	Before Experiment	After Experiment	
		Treated Set	Control Set
Total acid . .	1 gm = 11 04 c c. NaOH	1 gm = 12 6 c c NaOH	1 gm. = 10 5 c.c. NaOH
pH	4·04	4·2	4 1
Monosacchandes .. .	2·6%	5·4%	4·2%
Disacchandes	1·9%	3·5%	4·01%

Experiment XII.—Mangoes selected were 100–110 days old and a mixture of ethylene (0·1%), sulphur dioxide (0·01%), and air, was used. The respiratory rate of the treated fruit increased slightly at first, but greatly afterwards (Fig. 12). After the experiment the treated fruit was soft and



Control - - - - - - - Treated ————

FIG 12

pulpy with a light green skin; epicarp had become loose, mesocarp soft, pulpy and much disorganized with the lower portion brownish in colour. Sugar and acid contents were as follows:

TABLE X

	Before Experiment	After Experiment	
		Treated Set	Control Set
Total acid ..	.. 1 gm.= 11 4 c.c NaOH	1 gm = 10·2 c c NaOH	1 gm = 10·7 c c. NaOH
Monosaccharides .	6·3%	8·6%	6·27%
Disaccharides ..	.. 8·2%	9·1%	8·6%

Experiment XIII.—In this experiment injured mangoes of different ages were taken from the same tree and analysed for sugar and acid contents; the values for uninjured mangoes, given in the following table, have been taken from the previous experiments.

TABLE XI

Age in days	30		40		50		60		70	
Condition of the fruit	Injured	Un-injured	Injured	Un-injured	Injured	Un-injured	Injured	Un-injured	Injured	Un-injured
Total acid c c N/100 NaOH	8·2	6·7	8·7	8 1	9 09	9·04	9·7	10·01	11·5	10·7
pH	5 6	4 9	5·01	4 78	4 78	4 04	4 6		5 0	
Monosachharides gm %	2·4	1·32	2·8	1 4	3·58	2 6	4 6	3·6	6 7	3·6
Disaccharides gm %	1 6	1·1	2·65	1 2	3·8	1·9	5·9	3·1	11·8	6·8

Experiment XIV.—Injured mangoes were picked from the same tree at different periods. The fruits had yellow instead of black patches on them. The sugar and acid values of these were compared with gas treated mangoes of the laboratory, the values of which have been taken from previous records.

TABLE XII

(*Comparison of injured mangoes from tree: from lab.*)

Age in days	40		50		70–80	
Samples from	Tree	Lab	Tree	Lab	Tree	Lab
Total acid c c N/100 NaOH	9·09	8·7	10·0	9·09	10·0	11·5
Monosaccharides gm. %	2 65	2 8	4 3	3·58	5 2	6·7
Disaccharides gm %	2 3	2·65	5 3	3 8	9 8	11·8

Experiment XV.—In this experiment, equally old uninjured mangoes and mangoes with black tips were taken. They were separately cut into pieces, weighed, and left in an electric drying oven. They were weighed at intervals till their weights became constant.

Water content of injured fruit $= 80 \cdot 3\%$
Water content of uninjured fruit $= 74 \cdot 2\%$.

Experiment XVI.—In this experiment two mangoes were selected from an uninjured tree and one of these was placed in a chamber through which air, free of carbon dioxide, was passed. The other was placed in another chamber through which ethylene-air mixture (1 : 1,000) was passed. The experiment was continued for 10 days. On the 9th day, it was found that mangoes in ethylene had developed light brown patches on the skin, and on the 10th day the colour became darker. Plate XI shows the black patches on the mango.

On repeating the experiment with (1 : 500) ethylene-air mixture, browning occurred on the 7th day.

Experiment XVII.—The same experiment was performed with 0·1% sulphur dioxide instead of ethylene. Here no blackening occurred, but the skin turned whitish, through bleaching

Experiment XVIII.—Four mangoes were taken from an uneffected tree and two of them were placed in a chamber through which ethylene (0·1%), sulphur dioxide (0 01%)-air mixture was passed. The other two were placed in a chamber through which air free from carbon dioxide was passed. In the case of the gas-treated mangoes, blackening at the distal region occurred on the 6th day, and by the end of the 10th day the fruits began to deteriorate, the black area having become quite prominent.

Discussion of the Results

1. *Effect of Ethylene on Respiration.*—It is clear from the foregoing experiments that ethylene accelerates the respiratory rate of magnoes. It should be noticed, however, that the rate is not accelerated immediately after introducing ethylene, but after some time. This interval may be termed *induction period.* It will be seen from the various experimental records that the period varies in different cases, but, in no case, it is less than 15 hours. It is quite possible that the age of the fruit has some relation with the variations in the induction period in different cases; in Experiment I the mangoes were the youngest and the induction period was the longest. Another point in this connection is the diffusibility of the gas through the skin of the fruit; it is possible that the permeability of the skin varies with the age of the fruit, and consequently also the diffusion of ethylene through the skin. From this point of view Experiments V and VI are significant; in both these experiments mangoes of approximately the same age were employed, with the difference that in Experiment VI the skin of the mangoes was peeled off before the experiment was started. The effect of ethylene gas was felt 6 hours earlier in this set.

It has been stated above that ethylene accelerates the respiratory rate of mangoes. The acceleration is sudden, and the respiratory rate remains at a high level but has a tendency to fluctuate. It will be seen from records that acceleration is greatest in the case of mangoes 40–50 days old, *i.e.*, neither young nor very old. It will also be seen that the respiratory rate of the control set is higher at this stage. If respiratory rate is taken as a measure of metabolic flux it will be apparent that mangoes 40–50 days old are physiologically more active; it is a matter of course therefore, that the acceleration at this stage should be highest.

2. *Effect of Ethylene on Sugar Content.*—In Fig. 13 we have given in graphical form the quantities of the mono- and the disaccharides of the

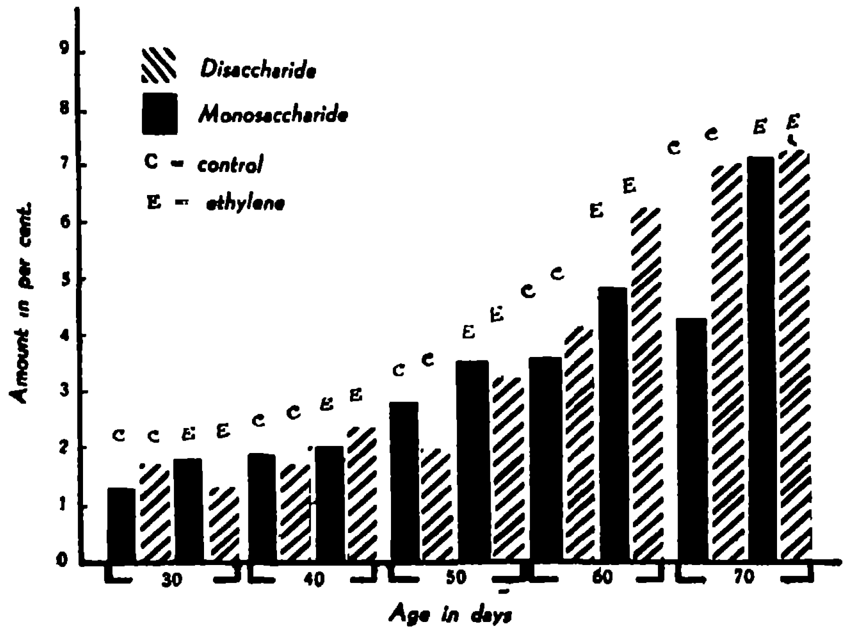


FIG. 13

control and ethylene treated fruits of various ages, taken from previous experiments. Two things come out prominently, *viz.*, that (1) the sugars tend to increase both in the control and ethylene treated fruits as they advance in age; (2) both the monoses and the dioses show a greater increase in the ethylene-treated fruits than in the control. This is especially so when the fruits are more advanced in age.

The increase of both the monoses and the dioses with the advancing age and also with ethylene treatment shows that the dioses are not the storage

carbohydrates, for if it were so then the monoses could have increased at the expense of the dioses. This naturally leads us to conclude that both these sugars are but the intermediate sugars formed from some other chemical substances within the fruit. And, if we take the increase of sugars as one of the criterions of ripening, we may safely conclude that ethylene treatment hastens the process. In normal cases, ripening takes place between 80 and 90 days while in treated fruits it takes place between 60 and 70 days. When fruits of earlier ages are treated then as seen from Fig. 13 the sugars do not increase appreciably, but instead disorganization of the cells sets in.

3. *Effect of Ethylene on pH and Total Acid.*—In several cases pH and total acid content of mangoes were determined. In the case of pH, no appreciable difference could be found between the treated and control sets. This is as should be expected since change in the pH involves a fundamental change in the system which is protected by buffer solutions. In the total acid content also not much difference is noted between the two sets, whatever little difference exists, is found in cases where older mangoes were selected for experiments; it must be admitted, however, that the difference is not in any way convincing.

4 *Effect of Sulphur Dioxide.*—It is evident from Experiments VIII and IX that sulphur dioxide also accelerates the respiratory rate of mangoes. In the case of this gas as well, the effect is not immediately felt, *i e.*, there is a period of induction. As seen in Experiment X the induction period was shortened by full 6 hours when the skin was peeled off.

5. *Comparison of the Effects of Ethylene and Sulphur Dioxide* —The effects of the two gases can be compared from two points of view : (1) the acceleration of the respiratory rate, and (2) the condition of the fruit after treatment. It will be noticed that the maximum effect of ethylene has been recorded in Experiment III; if the respiratory values, in this experiment, are compared with those in Experiments VIII or IX, it is evident that sulphur dioxide has greater accelerating effect, because ethylene at that age of the mangoes would have produced, at the same concentration as sulphurdioxide, less effect as experimental records (Exps. III–V) show a progressive decline in the increase of respiration in ethylene with advancing age of the fruit.

As regards the general condition, the mangoes treated with ethylene generally became soft and yellow, but they never deteriorated. On the other hand, those treated with sulphur dioxide decomposed. It is concluded, therefore, that sulphur dioxide has more adverse effect on mangoes than ethylene,

6. *Effect of Ethylene, Sulphur Dioxide and Air Mixture*.—The sugar values in all cases of treatment with ethylene-air mixture and also with ethylene-sulphur dioxide-air mixture are greater than their respective controls. But on comparing the sugar values of Experiments III and XI, it is found that mangoes treated with ethylene-sulphur dioxide-air mixture contain more sugar than those treated with ethylene alone. Ranjan and Zafar Ali[9] showed that the percentage of sugar in guava increased with the maturity and ripening of the fruit. From this point of view it will be seen that ethylene-sulphur dioxide-air mixture tends to produce physiological ripening of the mango fruit in greater degree than ethylene-air mixture. Ranjan and Zafar Ali[9] also showed that the acid values increase with the age and ripening of the fruit. It must be admitted here, however, that the corresponding acid values in the case of mangoes are not so convincing. The general condition of the fruits after the various treatments differ a great deal in the two cases, and it seems that a mixture of ethylene-sulphur dioxide-air mixture is more effective in bringing about physiological ripening than ethylene-air mixture.

7. *The Cause of Injury to the Mango Fruits near Brick-kilns*.—In tracing the real cause of the black tip injury one has to consider several probable causes. One of these is that the causative agency may be a living organism, the other, that the disease may be due to some physiological deficiency or to an unfavourable environment. The first of these is ruled out in view of the fact that careful examination did not reveal the presence of any micro-organism; moreover, if a micro-organism was present at all, it would not have been possible to find one of the adjacent fruits diseased and the other healthy, for infection in that case would have easily spread to neighbouring fruits. The idea of physiological deficiency is ruled out in view of the fact that the trees, to all appearances, were unaffected. Furthermore, such a condition of fruits has always been noticed in trees situated near brick-kilns. This suggestes some possible connection between environment and the damage, *i.e.*, between the brick-kilns and the black tip disease. That such is actually the case is clear from the results of Experiments XVI and XVIII. In Experiments XVI and XVIII, ethylene was administered and as a result, deep brown patches appear on the treated mangoes. In Experiment XVII only sulphur dioxide and air was given, and this resulted in the bleaching of skin colour of the fruits. The influence of gases, derived from a general survey of experiments recorded, shows, that mangoes were always softened by them and that, in a majority of cases, there was a corresponding change of skin colour from dark green to yellowish green. In nearly all the treated cases the mesocarp and even the epicarp was softened as a result of the action of the gases; it is to be recorded however, that the distal part softened in a greater measure

and that maximum external changes took place in the case of fruits treated with a mixture of ethylene, sulphur dioxide and air.

Now, if yellowness of the skin and pulpiness of external tissues, together with the increase of sugars, be taken as an index to the ripeness of a fruit, then the inevitable conclusion is that mangoes ripen much earlier in ethylene-sulphur dioxide-air mixture than in air alone. It was in the case of ethylene-air mixture and ethylene-sulphur dioxide-air mixture that cases of black spot occurred. But in sulphur dioxide, instead of a black spot, a general yellowness of the fruit took place. It must be pointed out that age is not a criterion for the black spots, because both young and old fruits were affected by the disease in the presence of ethylene alone or ethylene and sulphur dioxide together.

Summary

1. The physiology of the black tip disease of mangoes (*Mangifera indica*) has been studied with respect to ethylene, and sulphur dioxide, the two important and harmful constituents of blast furnace gas

2. The respiratory rate, sugar and acid contents of 30–100 days old mangoes have been studied. It was found that—

(*a*) Ethylene-air mixture accelerated after an induction period of 15 hours or more, depending on the age of the fruit, the respiratory rate and increased the sugar content, but it did not affect the total acid content of the fruit. Mangoes treated with ethylene generally became soft and yellow;

(*b*) The induction period was very much decreased if the skin was peeled off before the experiment;

(*c*) Prolonged treatment with a strong concentration of ethylene gas produced the characteristic black-tip disease of the mango;

(*d*) Sulphur dioxide-air mixture also had an induction period and accelerated the respiratory rate, but had an adverse effect on the general condition of mangoes;

(*e*) Ethylene-sulphur dioxide-air mixture increased the respiratory rate and sugar content, but made the mesocarp, especially its lower half, soft, pulpy and disorganized, and produced the black-tip disease of mangoes.

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The arrow indicates the black patch

Mangoes of different ages, near brick-kiln, showing black patches on them

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